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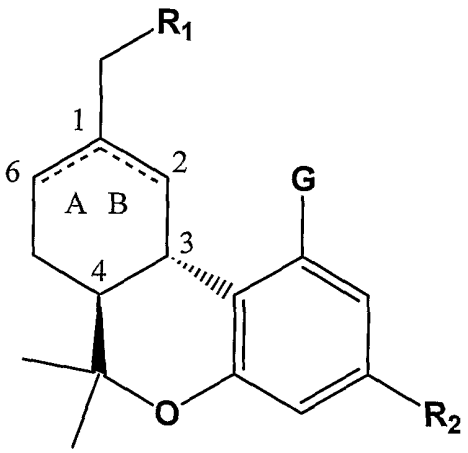
(54) Title: NOVEL NON-PSYCHOTROPIC CANNABINOIDS

(57) Abstract: Novel non-psychotropic cannabinoids are disclosed and pharmaceutical compositions comprising these novel compounds are described for preventing neurotoxicity, neuroinflammation, immune or inflammatory disorders comprising as active ingredient the stereospecific (+) enantiomer, having (3S, 4S) configuration of Δ^6 tetrahydrocannabinol type compounds. The compositions are particularly effective in alleviating and even preventing neurotoxicity due to acute injuries to the central nervous system, including mechanical trauma, compromised or reduced blood supply as may occur in cardiac arrest or stroke, or poisonings. They are also effective in the treatment of certain inflammatory disorders and chronic degenerative diseases characterized by neuronal loss and chronic pain including neuropathic pain.

NOVEL NON-PSYCHOTROPIC CANNABINOIDS

FIELD OF THE INVENTION

The present invention relates to a family of novel non-psychotropic cannabinoids, and to pharmaceutical compositions containing them, which are useful for preventing or alleviating neurotoxicity and inflammation. Said pharmaceutical compositions comprise as their active ingredient the stereospecific (+) enantiomers, having (3S, 4S) configuration, of Δ^6-tetrahydrocannabinol (THC) type compounds of general formula (I), as defined hereinbelow.



BACKGROUND OF THE INVENTION

The identification of tetrahydrocannabinol (THC) as the active principle of marijuana (Cannabis sativa) prompted medicinal chemists to develop numerous cannabinoid analogs (reviewed by Barth, *Exp. Opin. Ther. Patents* 8:301-313, 1998). These novel compounds were designed to exhibit the beneficial properties of THC without the accompanying psychotropic effects, which limit its therapeutic utility. Potential therapeutic applications have classically included known attributes of marijuana itself such as anti-emesis, analgesia, anti-glaucoma and appetite stimulation.

More recently recognized roles for non-psychotropic cannabinoids are as neuroprotective and anti-inflammatory agents.

Neuroprotective Activity

Chronic degenerative changes, as well as delayed or secondary neuronal damage following direct injury to the central nervous system (CNS), may result from pathologic changes in the brain's endogenous neurochemical systems. Although the precise mechanisms mediating secondary damage are poorly understood, post-traumatic neurochemical changes may include overactivation of neurotransmitter release or re-uptake, changes in presynaptic or postsynaptic receptor binding, or the pathologic release or synthesis of endogenous factors. The identification and characterization of these factors and of the timing of the neurochemical cascade after CNS injury provides a window of opportunity for treatment with pharmacologic agents that modify synthesis, release, receptor binding, or physiologic activity with subsequent attenuation of neuronal damage and improvement in outcome. A number of studies have suggested that modification of post-injury events through pharmacologic intervention can promote functional recovery in both a variety of animal models and clinical CNS injury. Pharmacologic manipulation of endogenous systems by such diverse pharmacologic agents as anticholinergics, excitatory amino acid antagonists, including specifically NMDA receptor antagonists, endogenous opioid antagonists, catecholamines, serotonin antagonists, modulators of arachidonic acid, antioxidants and free radical scavengers, steroid and lipid peroxidation inhibitors, platelet activating factor antagonists, anion exchange inhibitors, magnesium, gangliosides, and calcium channel antagonists have all been suggested to potentially improve functional outcome after brain injury (McIntosh, *J. Neurotrauma* 10:215-243, 1993).

The pathogenesis of a diverse group of neurological disorders has been linked to excessive activation of excitatory amino acid receptors. These disorders include epilepsy, focal and global ischemia, CNS trauma, and various forms of neurodegeneration including Huntington's chorea, Parkinson's disease and Alzheimer's disease. There has been extensive effort invested in the development of excitatory amino acid receptor antagonists as therapeutic agents (Rogawski, *Trends in*

Pharmacol. Sci. 14:325-331, 1993 and Danbolt, *Progress in Neurobiology* 65:1-105, 2001).

Since no proven effective therapy for neuronal injury, or degeneration, is yet known, and, for example, stroke alone is one of the leading causes of death in many countries, the importance of finding such therapeutic excitatory amino acid antagonists including specifically NMDA antagonists is self-evident. It will be important to determine whether certain NMDA receptor antagonists are more effective - or have fewer side effects - than others in specific disease states.

Some of the compounds of general Formula (I) are disclosed in U.S. Patents Nos. 4,179,517 and 4,876,276. As disclosed in said U.S. patents, these essentially pure synthetic (+)-(3S,4S)-THC derivatives and analogues are devoid of any undesired cannabimimetic psychotropic side effects. These known compounds have been described as having analgesic, antiemetic and anti-glaucoma activity.

A particular compound of interest of Formula I, is 1,1 dimethyl heptyl-(3S,4S)-7-hydroxy-Δ^6-tetrahydrocannabinol, disclosed in US 4,876,276, and denoted therein as HU-211, and subsequently assigned the trivial chemical name dexanabinol. HU-211 was unexpectedly discovered to possess neuroprotective attributes, which may be ascribed to its activity as a non-competitive antagonist at the NMDA receptor, as disclosed in US patents nos. 5,284,867 and 5,521,215. Certain ester derivatives of dexanabinol are also active in neuroprotection, as disclosed in US patent no. 6,096,740 as are the carboxylic acid derivatives of HU-211 as disclosed in US 5,538,993 and 5,635,530.

Anti-Inflammatory Activity

Besides NMDA receptor blocking activity, dexanabinol and its ester derivatives were further shown to possess anti-oxidative and anti-inflammatory properties, which may contribute to their efficacy in preventing or alleviating ischemic damage to tissues.

In addition, derivatives of HU-211(dexanabinol) were surprisingly shown to possess immunomodulatory potential due to their ability to inhibit Tumor Necrosis Factor alpha as disclosed in US 5,932,610.

Certain natural non-psychotropic cannabinoids, including the derivative cannabidiol, have been found to have antioxidant properties unrelated to NMDA receptor antagonism as disclosed in WO 99/53917.

Endogenous ligands of the cannabinoid receptors (Mechoulam, et al., *Eur. J. Pharmacol.* 359:1-18, 1998) have been identified as being arachidonyl derivatives including 2-arachidonyl glycerol, and arachidonyl-ethanolamide (anandamide). Thus, these endocannabinoids are chemically related to certain metabolites in the arachidonic acid pathway.

A family of compounds known to exhibit inflammatory properties is the prostaglandins (PG). Prostaglandins are arachidonic acid metabolites, produced by the action of cyclooxygenase (COX) also known as PGH synthase. Two isoforms of COX have been identified, COX-1 and COX-2. Although both perform the same catalytic activity they differ in tissue distribution, regulation and expression (Williams and DuBois *Am J Physiol.* 270:G393-400, 1996).

COX-1 is constitutively expressed and appears to be involved in the physiological production of PGs. Although COX-2 has a normal pattern of expression in some body tissues it is primarily an inducible form that is expressed upon prolonged exposure to chemical mediators including cytokines and endotoxin (reviewed in Golden and Abramson, *Osteoarthritis* 25:359-378, 1999) Pain and inflammation in certain pathological processes are mediated by the COX-2 dependent production of PGE_2. There is considerable interest in developing anti-inflammatory therapeutic strategies that block the activity of COX-2 and the biosynthesis of PGE_2 resulting from activation of the Arachidonic acid/prostaglandin (AA/PG) biosynthetic pathway.

Attenuation of COX2 activity is correlated with a reduction in pain, inflammation and fever. For example, the NSAIDs (non-steroidal anti-inflammatory drugs) act by blocking the COX enzymes. A reduction of 40-50% in the colon cancer rate among cardiovascular patients in the US who are given prophylactic doses of aspirin (a common NSAID) was also shown to be related to a decrease in COX-2 expression (Smalley and DuBois, *Adv Pharmacol* 39:1-20, 1997).

Therapeutic strategies that target this pathway are sought to prevent and treat a variety of diseases and symptoms such as neuronal degeneration in diseases as Alzheimer's disease or Parkinson's disease, neuronal trauma associated with seizures, brain or CNS damage, inflammation associated with rheumatoid arthritis; bone resorption and colonic polyposis and colorectal cancer (reviewed in Lipsky, *J Rheumatol* 26: Suppl 56:25-30, 1999). US Patent no. 5,840,746 teaches the method of

treating neurodegenerative disease by administering non-steroidal COX-2 inhibitors that specifically bind to COX-2. Inflammation has also been implicated as part of the pathogenesis in myocardial infarction, atheroma, unstable angina and other cardiac disorders (Ross, *New England J Med* 340:115-126, 1999).

There is an unmet need for and it would be advantageous to have novel non-psychotropic cannabinoid compounds that exert effects via a plurality of mechanisms. Ideally, in addition to having said analgesic, antiemetic and anti-glaucoma activities, they would also be effective against the diseases and conditions mentioned above. The mechanisms involved in these pleitropic effects include their action as excitatory amino acid receptor blockers, for example NMDA-receptor or glutamate-blockers or interaction with the glycine receptor, or as inhibitors of either the oxidative, cytokine, nitric oxide or AA/PG pathways, including the cyclooxygenase and lipoxygenase pathways.

It is explicit that the present invention excludes known compounds disclosed in US patents 4,876,276, 5,538,993, 5,635,530 and 6,096,740.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide pharmacologically acceptable non-psychotropic cannabinoids. It is a further object of the present invention to provide agents that can afford neuroprotection by exhibiting anti-inflammatory activity, and/or antioxidative activity, and/or the capacity to block the cyclooxygenase or lipoxygenase pathway, or the nitric oxide or cytokine pathways and/or to block excitatory amino acid mediated toxicity by interaction at specific receptors, such as glutamate receptors. It is yet a further object of the present invention to provide agents that can afford neuroprotection by combined anti-inflammatory, antioxidative and/or glutamate–receptor blocking mechanisms of action.

It is another object of the present invention to provide pharmaceutical compositions comprising as an active ingredient a non-psychotropic cannabinoid. The compositions will be useful for the treatment or prevention of ischemia in the CNS as well as in other tissues such as kidney, lung, liver, heart and joints. The compositions

will be neuroprotective and will be useful for the prevention or treatment of neurodegenerative disease as well as for glaucoma, pain, inflammation, and emesis.

The present invention discloses novel compounds and compositions that are effective in the alleviation and treatment of many of the abnormal states involving inflammation and toxicity. It will be noted that the compounds of the present invention may operate via diverse mechanisms to provide the neuroprotective and/or anti-inflammatory properties.

Certain embodiments of the present invention are particularly effective in alleviating and even preventing neurotoxicity due to excitatory amino acids, also referred to as glutamate neurotoxicity. Glutamate neurotoxicity may occur during acute injuries to the central nervous system (CNS), such as injuries due to prolonged seizures, compromised or reduced blood supply, deprivation of glucose supply and mechanical trauma. The present compositions are also effective in alleviating other damages to the CNS like damage resulting from poison-induced convulsions, including but not limited to those considered to be associated with amino acid receptors other than that of glutamate, for example glycine. Unexpectedly, neuroprotection is also a feature of some of the novel compounds that do not have a high affinity for the NMDA receptor.

The compositions of the present invention may also be effective in the treatment of certain chronic degenerative diseases that are characterized by gradual selective neuronal loss. In this connection, the compositions of the present invention are contemplated as therapeutically effective in the treatment of Alzheimer's disease, Parkinson's disease, Huntington's disease and amyotrophic lateral sclerosis. Surprisingly, we have shown experimentally that more preferred embodiments of this group of compounds can even promote nerve regeneration.

The present compositions are of special value in global hypoxic ischemic insults, in hypoxia, alone or in combination with blood flow reduction, such as cardiac, unstable myocardial, renal and hepatic ischemias, as well as in cases of cardiac arrest and in cases of abrupt occlusion of cerebral arteries (stroke).

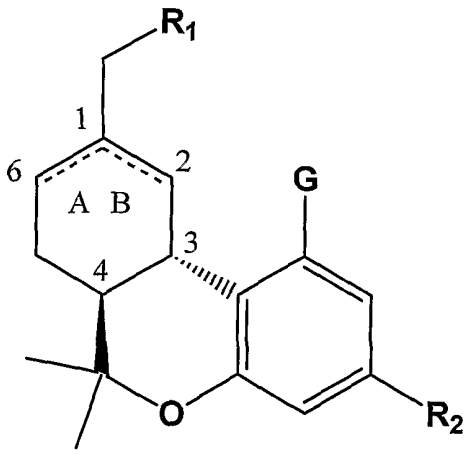
The present compositions are also particularly useful as analgesics, a known attribute of this class of compounds. The present compositions are also of special

value in inflammatory or immune diseases of the nervous system, exemplified by multiple sclerosis and other autoimmune diseases, encephalitis and HIV-induced neurodegeneration; arthritis such as rheumatoid arthritis and other types of inflammation; the cardiovascular system, exemplified by myocardial infarction, coronary heart disease, restenosis of coronary vessels and myocarditis; and of the pulmonary system, exemplified by asthma or chronic obstructive pulmonary disease (COPD).

A further object of the present invention provides compositions that can inhibit the AA/PG signaling pathways that regulate or are regulated by COX-2, an example being the prevention or treatment of the occurrence or growth of gastrointestinal tumors such as colorectal cancer and colonic polyps.

The therapeutic agents of the present invention comprise novel derivatives of non-psychotropic cannabinoids.

A first embodiment of the present invention provides novel compounds according to formula (I):



having the (3S,4S) configuration and being essentially free of the (3R,4R) enantiomer, wherein A-----B indicates an optional 1(2) or 6(1) double bond,

R_1 is

A) R3 where R3 is selected from the group consisting of

a) a linear or branched, saturated or unsaturated, carbon side chain comprising 1-8 carbon atoms interrupted by 1-3 heteroatoms; or

b) a saturated or unsaturated cyclic moiety, an aromatic moiety or a heterocyclic moiety; the cyclic moiety having from 5-20 atoms comprising one or two-ringed structures, wherein each ring comprises 3-8 carbons, interrupted by 1-4 heteroatoms, said heteroatoms each independently selected from the group consisting of N, O, and S; wherein each ring optionally is further substituted with one or more groups selected from

i) C_{1-6} alkyl,
ii) C_{1-6} alkoxy,
iii) C_{1-6} alkylthio,
iv) Halo,
v) Carboxyl
vi) $-CO_2-C_{1-4}$ alkyl
vii) keto,
viii) nitro,
ix) a saturated or unsaturated cyclic moiety, an aromatic or a heterocyclic moiety as defined in b above;

B) an amine or an amide substituted with at least one substituent as defined in R3 above;

C) a thiol, a sulfide, a sulfoxide, a sulfone, a thioester or a thioamide optionally substituted with one substituent as defined in R3 above;

D) an ether –OR3 wherein R3 is as defined above;

G is (a) halogen, (b) C_1-C_6 alkyl, or (c) -OR wherein R is (a') -R", wherein R" is hydrogen or C_1-C_6 alkyl optionally containing a terminal -OR'" or -OC(O)R'" moiety wherein R'" is hydrogen or C_1-C_6 alkyl, or (b') -C(O)R'" wherein R'" is as previously defined,

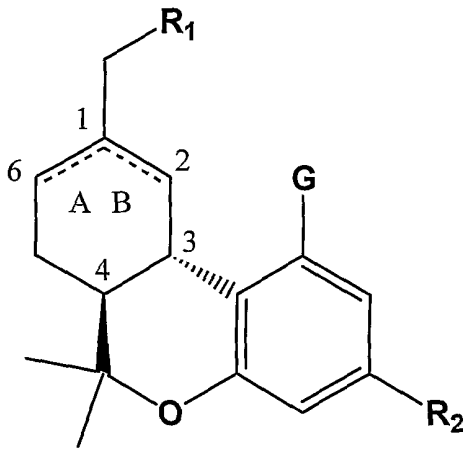
and $\mathbf{R_2}$ is (a) C_1-C_{12} alkyl, (b) -OR"", in which R"" is a straight chain or branched C_2-C_9 alkyl which may be substituted at the terminal carbon atom by a phenyl group, or (c) $-(CH_2)_nOR'''$ wherein n is an integer of 1 to 7 and R'" is hydrogen or C_1-C_6 alkyl;

with the proviso that R_1 is other than a heterocyclic moiety having a labile hydrogen atom so that said moiety acts as a carboxylic acid analogue.

For purposes of this specification C_{1-6} alkyl, C_{1-6} alkoxy and C_{1-6} alkylthio are intended to include saturated and unsaturated linear, branched and cyclic structures.

Currently more preferred compounds are those wherein G is hydroxy or lower acyloxy and wherein R2 is dimethylheptyl.

The present invention further relates to pharmaceutical compositions for the purposes set out above, comprising as an active ingredient a compound of the general formula (I):



R_1 is

A) R3 where R3 is selected from the group consisting of

a) a linear or branched, saturated or unsaturated, carbon side chain comprising 1-8 carbon atoms interrupted by 1-3 heteroatoms; or

b) a saturated or unsaturated cyclic moiety, an aromatic moiety or a heterocyclic moiety; the cyclic moiety having from 5-20 atoms comprising one or two-ringed structures, wherein each ring comprises 3-8 carbons, interrupted by 1-4 heteroatoms, said heteroatoms each independently selected from the group consisting of N, O, and S; wherein each ring optionally is further substituted with one or more groups selected from

i) C_{1-6} alkyl,

ii) C_{1-6} alkoxy,

iii) C_{1-6} alkylthio,

iv) Halo,

v) Carboxyl

vi) $-CO_2-C_{1-4}$ alkyl

vii) keto,

viii) nitro,

ix) a saturated or unsaturated cyclic moiety, an aromatic or a heterocyclic moiety as defined in b;

B) an amine or an amide substituted with at least one substituent as defined in R3 above;

C) a thiol, a sulfide, a sulfoxide, a sulfone, a thioester or a thioamide optionally substituted with one substituent as defined in R3 above;

D) an ether –OR3 wherein R3 is as defined above;

G is (a) halogen, (b) C_1-C_6 alkyl, or (c) -OR wherein R is (a') -R", wherein R" is hydrogen or C_1-C_6 alkyl optionally containing a terminal -OR'" or -OC(O)R'" moiety wherein R'" is hydrogen or C_1-C_6 alkyl, or (b') -C(O)R'" wherein R'" is as previously defined,

and $\mathbf{R_2}$ is (a) C_1-C_{12} alkyl, (b) -OR"", in which R"" is a straight chain or branched C_2-C_9 alkyl which may be substituted at the terminal carbon atom by a phenyl group, or (c) $-(CH_2)_nOR'''$ wherein n is an integer of 1 to 7 and R'" is hydrogen or C_1-C_6 alkyl;

with the proviso that $\mathbf{R_1}$ is other than a heterocyclic moiety having a labile hydrogen atom so that said moiety acts as a carboxylic acid analogue.

For purposes of this specification C_{1-6} alkyl, C_{1-6} alkoxy and C_{1-6} alkylthio are intended to include saturated and unsaturated linear, branched and cyclic structures.

Currently more preferred compounds are those wherein G is hydroxy or lower acyloxy and wherein R2 is dimethylheptyl.

According to currently preferred embodiments of the present invention $\mathbf{R_1}$ is a heterocyclic moiety selected from the group consisting of an imidazolyl, an imidazolinyl, a morpholino, a piperidyl, a piperazinyl, a pyrazolyl, a pyrrolyl, a pyrrolidinyl, a triazolyl, and a tetrazolyl.

According to further currently preferred embodiments of the present invention $\mathbf{R_1}$ is a heterocyclic moiety selected from the group consisting of an imidazolyl, an imidazolinyl, a morpholino, a piperidyl, a piperazinyl, a pyrazolyl, a pyrrolyl, a pyrrolidinyl, a triazolyl, and a tetrazolyl, each cyclic moiety optionally further substituted wherein the substituent is selected from the group consisting of C_{1-6} alkyl, C_{1-6} alkyloxy, C_{1-6} alkylthio, keto, carboxy, nitro, saturated or unsaturated cyclic moieties or aromatic or heterocyclic moieties wherein each ring comprises 3-8 carbons interrupted by 1-4 heteroatoms, said heteroatoms in each independently selected from the group consisting of N, O, and S, wherein each ring optionally is further substituted with one or more groups selected from the group consisting of C_{1-6} alkyl, C_{1-6} alkyloxy, C_{1-6} alkylthio, keto , carboxy , or nitro.

According to more preferred embodiments of the present invention $\mathbf{R_1}$ is selected from the group consisting of imidazole, pyrazole, oxazole, isoxazole, tetrahydropyridine, pyrazoline, oxazoline, pyrrolidine, imidazoline, 2-thio-imidazole, 2-methylthio-imidazoline, 4-methyl-2-imidazoline, 4,4-dimethyl-2-imidazoline, methyl sulfide, methylsulfoxide, acetamido, benzamide, cyano, 1,2,4-triazole, 1,3,4-triazole, 1,2,3,4-tetrazole, 1,2,3,5-tetrazole, thiophene, phenyl, morpholine, thiomorpholine, thiazolidine, glycerol, piperazine, 4-piperidinopiperidine , 4-methylpiperidine and tetrahydropyran.

According to additional more preferred embodiments of the present invention $\mathbf{R_1}$ is selected from the group consisting of mono or di-substituted amines wherein the substituent is selected from the group consisting of C_{1-6} alkyl, C_{1-6} alkyloxy, C_{1-6} alkylthio, imidazolyl, an imidazolinyl, a morpholino, a piperidyl, a piperazinyl, a pyrazolyl, a pyrrolyl, a pyrrolidinyl, a triazolyl, and a tetrazolyl, wherein each cyclic structure may optionally be further substituted with at least one substituent selected from the group consisting of C_{1-6} alkyl, C_{1-6} alkyloxy, C_{1-6} alkylthio, keto , carboxy , or nitro, wherein C_{1-6} alkyl, C_{1-6} alkoxy and C_{1-6} alkylthio are intended to include saturated and unsaturated linear, branched and cyclic structures.

The scope of the present invention includes the use of the compounds and compositions for the treatment of neurological, neurodegenerative, inflammatory and ischemic disorders.

The invention further relates to methods of treatment comprising administering to a patient in need thereof, of a therapeutically effective amount of a composition comprising a compound according of the present invention.

The inventors have discovered that certain novel compounds of formula (I), which are currently most preferred active agents of the presently claimed compositions, are dexanabinol derivatives wherein R_1 is a heterocyclic moiety, which exhibit efficient anti-inflammatory properties, including inhibition of prostaglandin synthesis, as well as inhibition of tumor necrosis factor production, and inhibition of nitric oxide production, in addition to NMDA receptor blocking and anti-oxidative activity.

Unexpectedly, the inventors have discovered that certain novel compounds of formula (I), which are currently most preferred active agents of the presently claimed compositions, are dexanabinol derivatives wherein R_1 is a substituted amine as defined above which exhibit efficient anti-inflammatory properties, including inhibition of prostaglandin synthesis, as well as inhibition of tumor necrosis factor production, and inhibition of nitric oxide production, while being substantially inactive as NMDA receptor blockers.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 depicts the synthetic scheme used to synthesize certain preferred compounds designated PRS-211, using dexanabinol as the starting material.

Fig. 2 shows binding curves of compounds according to the present invention to the NMDA receptor, as measured by the displacement of labeled MK-801.

Fig. 3 shows TNF alpha inhibition by novel dexanabinol derivatives.

Fig. 4 illustrates inhibition of PGE2 by novel dexanabinol derivatives.

Fig. 5 shows nitric oxide synthase inhibition by novel dexanabinol analogs.

Figs. 6-8 Show the decreased mortality and improved clinical and neurological outcome following transient middle cerebral artery occlusion in rats treated with certain preferred dexanabinol derivatives.

Figs. 9-10 Show the ED 50 of certain preferred dexanabinol derivatives in inhibiting inflammation as assessed in the ear edema test.

Fig. 11 illustrates the improvement in contralateral performance in animals treated with certain preferred dexanabinol derivatives as assessed in the Staircase test.

Fig. 12 shows the change in cerebral infarct size in animals treated with certain preferred dexanabinol derivatives as assessed in the transient MCAo test.

Fig. 13 depicts the change in necrotic area in animals treated with certain preferred dexanabinol derivatives as assessed in the myocardial ischemia model.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides novel compounds belonging to the class of non-psychotropic cannabinoids, as well as pharmaceutical compositions comprising these compounds, and methods of using such compounds. Compounds of this class are effective agents for the treatment and prevention of emesis, glaucoma and pain and have been shown to possess neuroprotective and anti-inflammatory properties.

The compositions of the present invention are effective to reduce or even prevent neurological damage, including but not limited to excitatory amino acid neurotoxicity, due to acute injury or poisoning of the CNS, such as injuries due to prolonged seizures, compromised or reduced blood supply, deprivation of glucose

supply and mechanical trauma, and poisonings by, for example, strychnine, picrotoxin or organophosphorous compounds.

The compositions of the present invention may also be effective in treating certain chronic degenerative diseases that are characterized by gradual selective neuronal loss. In this connection, the compositions of the present invention are contemplated as therapeutically effective in the treatment of Huntington's chorea, amyotrophic lateral sclerosis, Parkinson's disease and Alzheimer's disease, via mechanisms of neuroprotection and/or nerve regeneration.

As stated above, the present compositions are of special value in seizures, global hypoxic ischemic insults, in hypoxia, alone or in combination with blood flow reduction (ischemia such as cardiac, unstable myocardial, pulmonary, renal and hepatic ischemias) as well as in cases of cardiac arrest and in cases of abrupt occlusion of cerebral arteries (stroke).

The compositions of the present invention are neuroprotective and may exert their neuroprotective actions through multiple mechanisms including, but not limited to anti-inflammatory and/or antioxidative mechanisms, and some of them are particularly effective in alleviating and even preventing glutamate neurotoxicity due to acute injuries to the central nervous system (CNS), such as injuries due to prolonged seizures, compromised or reduced blood supply, deprivation of glucose supply and mechanical trauma. The present compositions are also effective in alleviating other damages to the CNS like poison-induced convulsions, considered to be associated with amino acid receptors other than that of glutamate, for example glycine.

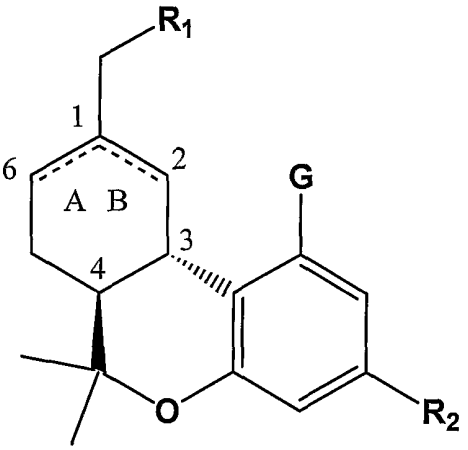
The compositions of the present invention are also effective in the treatment or prevention of pain, including chronic pain and neuropathic pain.

By virtue of their anti-inflammatory properties it will be recognized that the compositions according to the present invention will be useful in a wide variety of additional indications having an inflammatory or autoimmune mechanism involved in their etiology or pathogenesis exemplified by multiple sclerosis, arthritis such as rheumatoid arthritis and other types of local/general inflammation, encephalitis and HIV-induced neurodegeneration.

Another feature of the present invention is its ability to prevent or treat the occurrence or growth of gastrointestinal tumors such as colorectal cancer and colonic polyposis.

A set of the pharmaceutical compositions of the present invention exhibit inhibitory activity on NOS and cytokines as well as the AA/PG signaling pathways that regulate or are regulated by COX-2. The therapeutic agents of the present invention comprise novel derivatives of non-psychotropic cannabinoids.

The present invention relates to pharmaceutical compositions for the purposes



set out above, in which the active ingredient is a compound of the general formula (I):

having the (3S,4S) configuration and being essentially free of the (3R,4R) enantiomer, wherein A-----B indicates an optional 1(2) or 6(1) double bond,

R_1 is

A) R3 where R3 is selected from the group consisting of

a) a linear or branched, saturated or unsaturated, carbon side chain comprising 1-8 carbon atoms interrupted by 1-3 heteroatoms; or

b) a saturated or unsaturated cyclic moiety, an aromatic moiety or a heterocyclic moiety; the cyclic moiety having from 5-20 atoms comprising one or two-ringed structures, wherein each ring comprises 3-8 carbons, interrupted by 1-4 heteroatoms, said heteroatoms each independently selected from the group consisting of N, O, and S; wherein each ring

optionally is further substituted with one or more groups selected from wherein each ring optionally is further substituted with one or more groups selected from

i) C_{1-6} alkyl,
ii) C_{1-6} alkoxy,
iii) C_{1-6} alkylthio,
iv) Halo,
v) Carboxyl
vi) $-CO_2-C_{1-4}$ alkyl
vii) keto,
viii) nitro,
ix) a saturated or unsaturated cyclic moiety, an aromatic or a heterocyclic moiety as defined in b;

B) an amine $-N(R3)_2$ or an amide –N(R3)-COR3 substituted with at least one substituent as defined in R3 above;

C) a thiol –R3SH, a sulfide –SR3, a sulfoxide –SOR3, a sulfone SO_2R3, a thioester -SCOR3 or a thioamide –NC(S)R3 optionally substituted with one substituent as defined in R3 above;

D) an ether –OR3 wherein R3 is as defined above;

G is (a) halogen, (b) C_1-C_6 alkyl, or (c) -OR wherein R is (a') -R", wherein R" is hydrogen or C_1-C_6 alkyl optionally containing a terminal -OR'" or -OC(O)R'" moiety wherein R'" is hydrogen or C_1-C_6 alkyl, or (b') -C(O)R'" wherein R'" is as previously defined,

and $\mathbf{R_2}$ is (a) C_1-C_{12} alkyl, (b) -OR"", in which R"" is a straight chain or branched C_2-C_9 alkyl which may be substituted at the terminal carbon atom by a phenyl group, or (c) $-(CH_2)_nOR'''$ wherein n is an integer of 1 to 7 and R'" is hydrogen or C_1-C_6 alkyl;

with the proviso that $\mathbf{R_1}$ is other than a heterocyclic moiety having a labile hydrogen atom so that said moiety acts as a carboxylic acid analogue.

For purposes of this specification C_{1-6} alkyl, C_{1-6} alkoxy and C_{1-6} alkylthio are intended to include saturated and unsaturated linear, branched and cyclic structures.

Clearly it is intended that the heterocyclic structures are interrupted by the heteroatoms.

Currently more preferred compounds are those wherein G is hydroxy or lower acyloxy and wherein R2 is dimethylheptyl.

According to currently preferred embodiments of the present invention $\mathbf{R_1}$ is a heterocyclic moiety selected from the group consisting of an imidazolyl, an imidazolinyl, a morpholino, a piperidyl, a piperazinyl, a pyrazolyl, a pyrrolyl, a pyrrolidinyl, a triazolyl, and a tetrazolyl.

According to further currently preferred embodiments of the present invention $\mathbf{R_1}$ is a heterocyclic moiety selected from the group consisting of an imidazolyl, an imidazolinyl, a morpholino, a piperidyl, a piperazinyl, a pyrazolyl, a pyrrolyl, a pyrrolidinyl, a triazolyl, and a tetrazolyl, each cyclic moiety optionally further substituted wherein the substituent is selected from the group consisting of C_{1-6} alkyl, C_{1-6} alkyloxy, C_{1-6} alkylthio, keto, carboxy, nitro, saturated or unsaturated cyclic moieties or aromatic or heterocyclic moieties wherein each ring comprises 3-8 carbons interrupted by 1-4 heteroatoms, said heteroatoms in each independently selected from the group consisting of N, O, and S, wherein each ring optionally is further substituted with one or more groups selected from the group consisting of C_{1-6} alkyl, C_{1-6} alkyloxy, C_{1-6} alkylthio, keto , carboxy , or nitro.

According to more preferred embodiments of the present invention $\mathbf{R_1}$ is selected from the group consisting of imidazole, pyrazole, oxazole, isoxazole, tetrahydropyridine, pyrazoline, oxazoline, pyrrolidine, imidazoline, 2-thio-imidazole, 2-methylthio-imidazoline, 4-methyl-2-imidazoline, 4,4-dimethyl-2-imidazoline, methyl sulfide, methylsulfoxide, acetamido, benzamide, cyano, 1,2,4-triazole, 1,3,4-triazole, 1,2,3,4-tetrazole, 1,2,3,5-tetrazole, thiophene, phenyl, morpholine, thiomorpholine, thiazolidine, glycerol, piperazine, 4-piperidinopiperidine, 4-methylpiperidine and tetrahydropyran.

According to additional more preferred embodiments of the present invention $\mathbf{R_1}$ is selected from the group consisting of mono or di-substituted amines wherein the substituent is selected from the group consisting of C_{1-6} alkyl, C_{1-6} alkyloxy, C_{1-6} alkylthio, imidazolyl, an imidazolinyl, a morpholino, a piperidyl, a piperazinyl, a

pyrazolyl, a pyrrolyl, a pyrrolidinyl, a triazolyl, and a tetrazolyl, wherein each cyclic structure may optionally be further substituted with at least one substituent selected from the group consisting of C_{1-6} alkyl, C_{1-6} alkyloxy, C_{1-6} alkylthio, keto , carboxy , or nitro, wherein C_{1-6} alkyl, C_{1-6} alkoxy and C_{1-6} alkylthio are intended to include saturated and unsaturated linear, branched and cyclic structures.

In a currently preferred group of compounds, $\mathbf{R_2}$ designates a 1,1-dimethylalkyl radical or a 1,2-dimethylalkyl radical with a total of at least 7 carbon atoms. Also preferred are precursors of such compounds. Particularly preferred compounds are those wherein R_2 is 1,1-dimethylheptyl or 1,2-dimethylheptyl. It is these embodiments of R_2 that are found in THC and its most potent analogues. However, for the neuroprotective activity that characterizes the present invention, it is believed that any lower or mid-range alkyl substituent will be suitable at this position.

Throughout this specification, the compounds of the present invention may be referred to by their internal reference numbers rather than by their full chemical names. The prefix for this series of compounds is PRS-211, followed by a three-digit code for each specific compound of the series.

One currently most preferred compound, with which many of the physiological experiments have been carried out, is the compound, which may be referred to as (+)-(3S,4S)-6,6-Dimethyl-(1,1-dimethylheptyl)-1-hydroxy-9-(imidazolomethyl)-6a,7,10,10a- tetrahydro-6H- dibenzo[b,d]pyran. Said compound is designated hereinafter as PRS-211,095.

Another currently most preferred compound, with which many of the physiological experiments have been carried out, is the compound, which may be referred to as (+)-(3S,4S)-6,6-Dimethyl-(1,1-dimethylheptyl)-1-hydroxy-9-(pyrazolomethyl)-6a,7,10,10a- tetrahydro-6H- dibenzo[b,d]pyran. Said compound is designated hereinafter as PRS-211,220.

Another currently most preferred compound, with which many of the physiological experiments have been carried out, is the compound, which may be referred to as (+)-(3S,4S)-6,6-Dimethyl-(1,1-dimethylheptyl)-1-hydroxy-9-(1H-imidazol-2-ylsulfanyl methyl)-6a,7,10,10a- tetrahydro-6H- dibenzo[b,d]pyran. Said compound is designated hereinafter as PRS-211,092.

Another currently most preferred compound, with desirable PGE2 and NOS inhibitory activity, which may be referred to as (+)-(3S,4S)-6,6-Dimethyl-(1,1-dimethylheptyl)-1-hydroxy-9-(4-piperidinopiperidine methyl) 6a,7,10,10a- tetrahydro-6H- dibenzo[b,d]pyran. Said compound is designated hereinafter as PRS-211,257.

Another currently most preferred compound, with desirable PGE2 and NOS inhibitory activity, which may be referred to as (+)-(3S,4S)-6,6-Dimethyl-(1,1-dimethylheptyl)-1-hydroxy-9-(4-methylpiperidine methyl)-6a,7,10,10a- tetrahydro-6H- dibenzo[b,d]pyran. Said compound is designated hereinafter as PRS-211,251.

It is stressed that all the compounds are of the (+)-(3S,4S) configuration, essentially free of the (-)-(3R,4R) enantiomer, the latter known to possess the undesired psychotropic side effects. Thus, for example, the enantiomers of the synthetic cannabinoid 7-hydroxy-Δ^6-tetrahydrocannabinol 1,1-dimethylheptyl homolog, have been described [Mechoulam, R., *et al., Tetrahedron: Asymmetry* **1: 315-319,** 1990; Mechoulam, R. *et al., Experientia* **44: 762-764,** 1988]. The (-)-(3R,4R) enantiomer, herein designated HU-210, is a highly potent cannabimimetic compound (nearly 100 times more active than Δ-1-tetrahydrocannabinol, the active component of hashish). The (+)-(3S,4S) enantiomer, herein designated HU-211, also known by the trivial chemical name dexanabinol, while known to be active as an analgesic and as an anti-emetic, is inactive as a cannabimimetic even at doses several thousand times higher than the ED_{50} of HU-210 (Mechoulam, R. *et al., Experientia* **44: 762-764**, 1988).

As mentioned above, then, the compounds of the general formula (I) as defined herein are substantially devoid of cannabimimetic central nervous system activity.

All of the compounds of the present invention are stereospecific (+) enantiomers of the naturally occurring (-) cannabinoids. The (+) stereospecificity provides compounds that are devoid of psychotropic activity, and have been shown to have substantially no binding to the cannabinoid receptor CB1 of the central nervous system. The IC50 of binding of these novel compounds to the CB1 receptor is greater than 300 nM, more preferably greater than 1 μM, most preferably greater than 5 μM.

Pharmacology

The novel compositions contain in addition to the active ingredient conventional pharmaceutically acceptable carriers, diluents and the like. Solid compositions for oral administration such as tablets, pills, capsules or the like may be prepared by mixing the active ingredient with conventional, pharmaceutically acceptable ingredients such as corn starch, lactose, sucrose, sorbitol, talc, stearic acid, magnesium stearate, dicalcium phosphate and gums with pharmaceutically acceptable diluents. The tablets or pills can be coated or otherwise compounded with pharmaceutically acceptable materials known in the art to provide a dosage form affording prolonged action or sustained release. Other solid compositions can be prepared as suppositories, for rectal administration. Liquid forms may be prepared for oral administration or for injection, the term including subcutaneous, transdermal, intravenous, intrathecal, and other parenteral routes of administration. The liquid compositions include aqueous solutions, with or without organic cosolvents, aqueous or oil suspensions, flavored emulsions with edible oils, as well as elixirs and similar pharmaceutical vehicles. In addition, the compositions of the present invention may be formed as aerosols, for intranasal and like administration.

The active dose for humans is generally in the range of from 0.05 mg to about 50 mg per kg body weight, in a regimen of 1-4 times a day. However, administration every two days may also be possible, as the drug has a rather prolonged action. The preferred range of dosage is from 0.1 mg to about 20 mg per kg body weight. However, it is evident to the man skilled in the art that dosages would be determined by the attending physician, according to the disease to be treated, method of administration, patient's age, weight, contraindications and the like.

All the compounds defined above are effective as either NMDA-receptor blockers or oxidative or inflammatory pathway inhibitors and can be used as active ingredients of pharmaceutical compositions for treatment of one, or simultaneously several, symptoms of the disorders defined above. The effective dosages are essentially similar, and the more pronounced effect is that of NMDA-receptor blocking, in addition to the known characteristics of these compounds. However, it is important to note that the compounds and compositions of the present invention exhibit good blocking activity also against convulsants that may not necessarily be

NMDA-receptor mediators. For example, the compositions of the present invention can prevent, or at least alleviate, poisoning caused by strychnine organophosphorous compounds and nitrous oxide.

The compounds of the present invention are administered for the above-defined purposes in conventional pharmaceutical forms, with the required solvents, diluents, excipients, etc. to produce a physiologically acceptable formulation. They can be administered by any of the conventional routes of administration. The required dose for humans ranges from 0.005 mg/kg to about 50 mg/kg per unit dosage form. The most preferred dose range is from about 0.1 mg/kg to about 20 mg/kg body weight.

It will be appreciated that the most appropriate administration of the pharmaceutical compositions of the present invention will depend on the type of injury or disease being treated. Thus, the treatment of acute head trauma, stroke or ischemic brain damage resulting from cardiac arrest will necessitate systemic administration of the drug as rapidly as possible after induction of the injury. On the other hand, diminution or prophylaxis of chronic degenerative damage, inflammation or gastrointestinal cancer therapy will necessitate a sustained dosage regimen.

PRS-211 compounds convey significant neuroprotection in different in vivo models of head trauma, as well as transient and permanent brain ischemia. This suggests neuroprotective potential in a wide spectrum of CNS diseases, poisonings or injuries, as detailed above, including conditions involving axonal damage such as that sustained in spinal cord injury. PRS-211 compounds are also particularly useful in treating neural edema, associated with trauma, infection, tumors or surgical procedures including craniotomies and spinal cord manipulation.

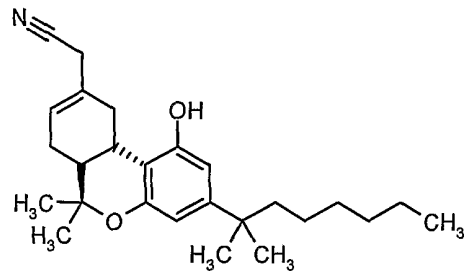
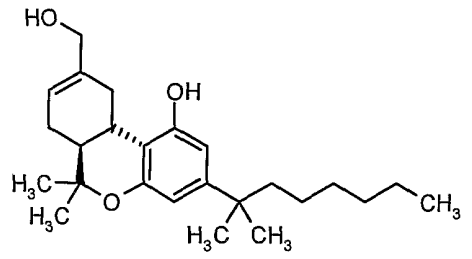
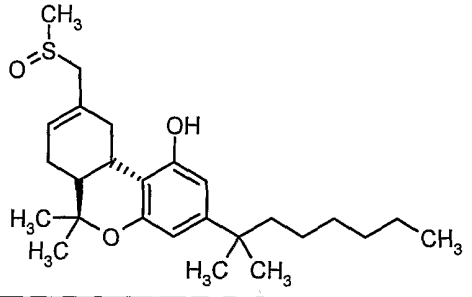
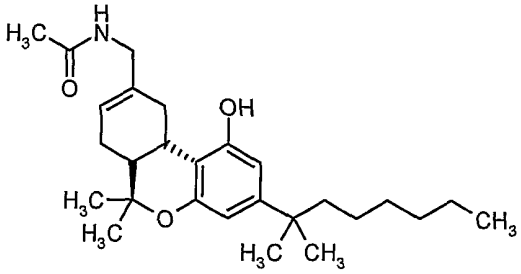
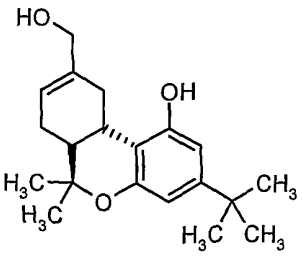
Moreover, the combined neuroprotective and anti-inflammatory properties of PRS-211 compounds, as well as the known anti-glaucoma properties of this class of compounds, leads to special consideration of retinal eye diseases, especially those which are associated with ischemic damage or a hostile biochemical environment. Some non-limiting examples would be diabetic retinopathy, age-related Macular Degeneration, retinal vascular occlusions that are relatively common and may cause considerable ischemic damage. All retinal occlusions, venous and arterial, including

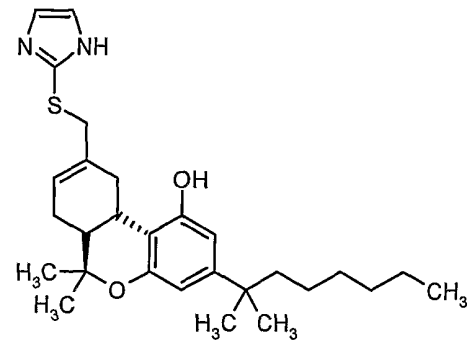
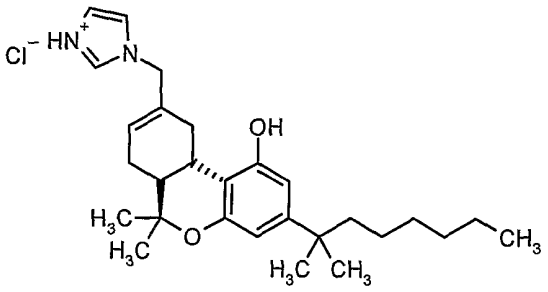
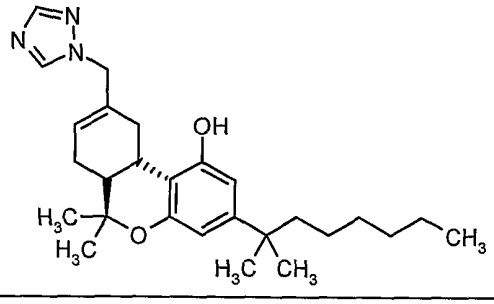
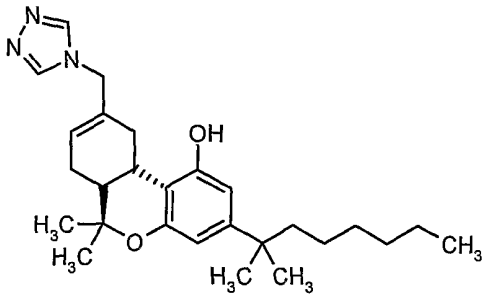
the optic nerve (ischemic optic neuropathy), as well as retinopathy of prematurity (oxygen toxicity in premature babies), may be included in this category, as well as any insult that may lead to secondary neural damage following direct retinal cell death, e.g. trauma, including surgical trauma such as laser burn injuries, inflammations, infections and degenerative processes, chronic ischemic damage, including glaucomatous optic nerve damage and toxic damage (e.g., chloroquine toxicity) and chronic malnutrition.

The inventors have discovered that certain novel compounds of formula (I), which are preferred active agents of the presently claimed compositions, such as PRS-211,092, PRS-211,095, PRS-211,128, PRS–211,132 PRS-211,220, PRS-211,251 and exhibit combined mechanisms of neuroprotection and anti-inflammation, including inhibition of prostaglandin and leukotriene synthesis, as well as the inhibition of nitric oxide synthesis (as measured by the inhibition of nitric oxide synthase (NOS))* and the production of cytokines such as tumor necrosis factor (TNFα) and interleukin-1β , in addition to the NMDA blocking and anti-oxidative activity.

Certain novel compounds of formula (I) such as the majority of the amine derivatives of PRS-211 such as PRS-211,251, PRS-211,253, PRS-211,255 or PRS-211,257 do not exhibit substantial NMDA receptor binding but exert their effect via inhibition of the AA/PG pathway and/or as anti-oxidatives. A tabulation of the more preferred compounds according the present invention, is presented in Table 1, whereas their variegated patterns of activities in terms of NMDA binding, anti-inflammatory and anti oxidative activities is presented in Table 2. These tables include the known compound, HU-211 (dexanabinol) for the sake of comparison.

Table 1: Chemical Structures of Novel PRS-211 compounds

	211,006-000
	211,007-000 HU-211
	211,041-000
	211,044-000
	211,047-000

	211,092-000
	211,095-000
	211,102-000
	211,103-000
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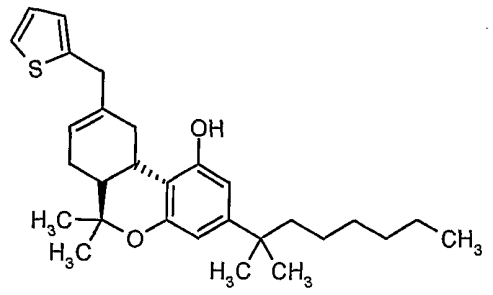
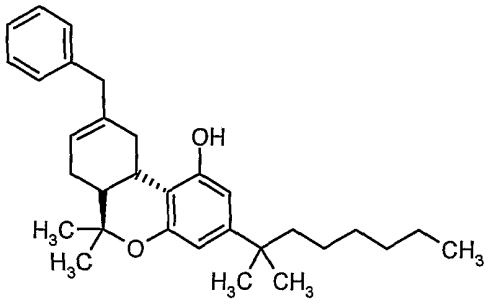
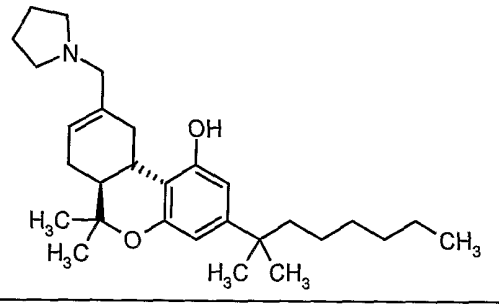
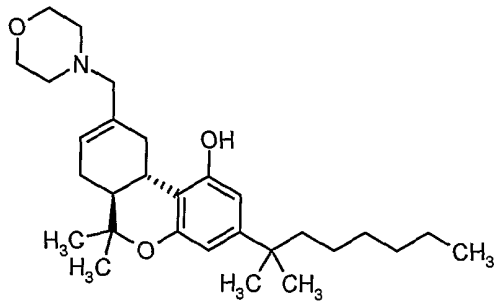
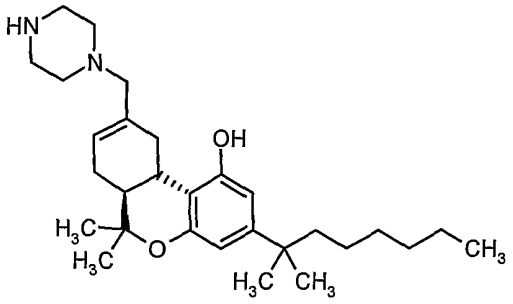
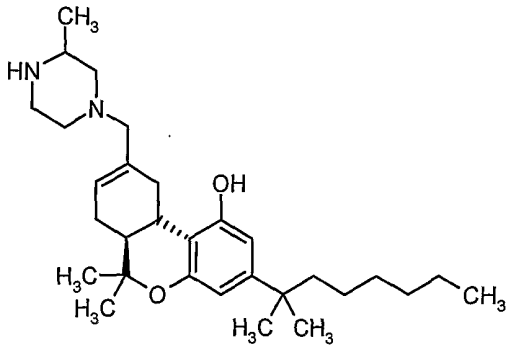
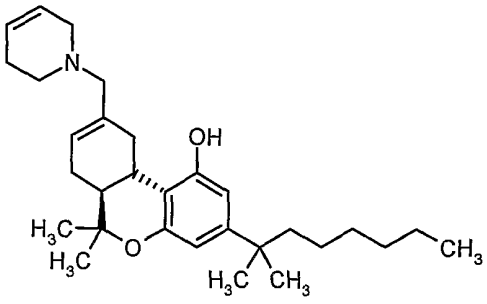
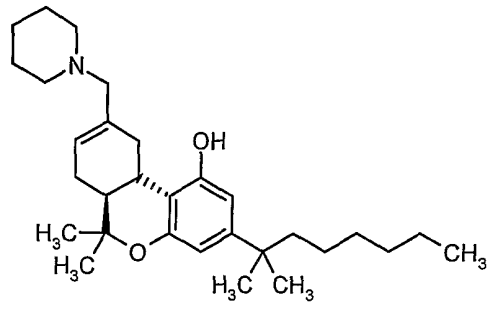
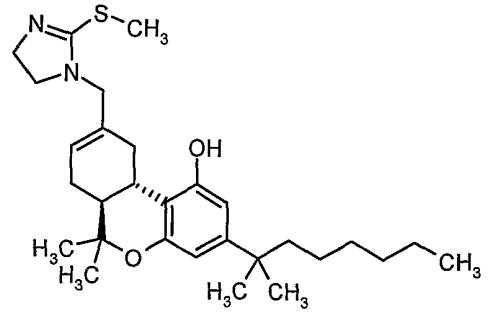
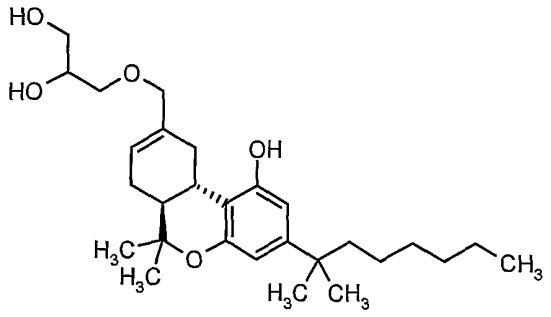
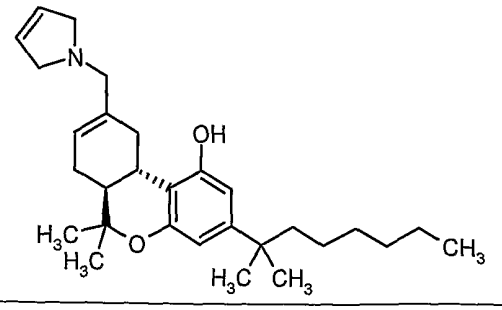
	
	211,119-000
	211,128-000
	211,132-000
	211,133-000

	211,134-000
	211,145-000

	211,159-000
	211,204-000
	211,211-000
	211,212-000

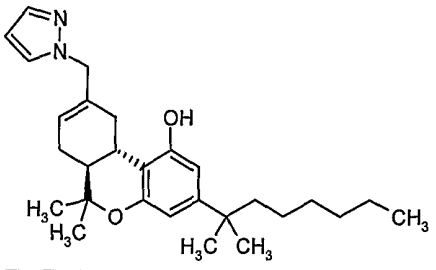
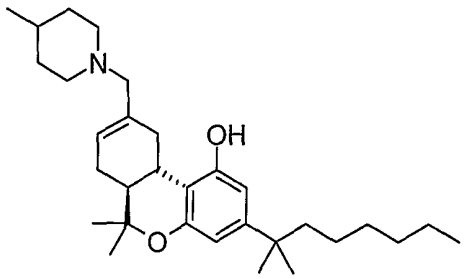
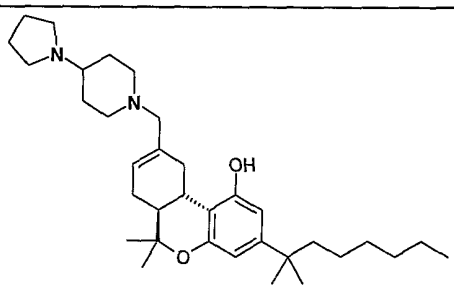
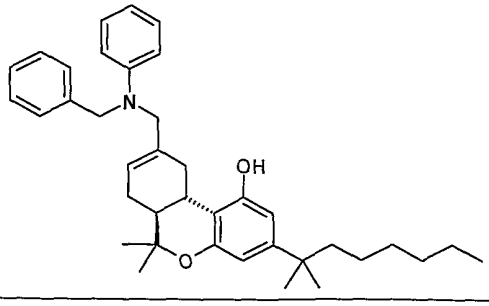
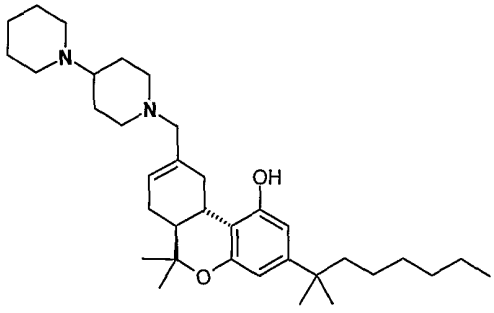
	211,220-000
	211,251-000
	211,253-000
	211, 255-000
	211, 257-000

Table 2. ACTIVITIES OF PRS-211 COMPOUNDS.

PRS number	NMDA BINDING IC50 (μM)	PGE2 % inhibition (at 10μM)	TNFα % inhibition (at 10μM)	NOS % inhibition (at 10μM)	Ear Edema ED50 (μmol/kg) CO
211,006-000	8	41	46	19	104
211,007-000 HU-211	10	54	33	20	25
211,041-000	6	56	23	72	39
211,044-000	6	24	0		
211,047-000	100	28	0		
211,092-000	>50	57	22	0	28
211,095-000	2.5	72	11	14	29
211,102-000	2.5				
211,103-000	3				
211,118-000	32				
211,119-000	62	14			
211,128-000	>20	84	48	27	23
211,132-000	4.5	61	21	40	70
211,133-000	> 100				
211,134-000	> 100				
211,145-000	13	63	15	60	
211,159-000	>20	87	33	27	
211,204-000	10	83	28	85	34
211,211-000	4.6	83	18	0	

PRS number	NMDA BINDING IC50 (μM)	PGE2 %inhibition (at 10μM)	TNF□ % inhibition (at 10μM)	NOS % inhibition (at 10μM)	Ear Edema ED50 (μmol/kg) CO
211,212-000	7.5	96	14	0	
211,220-000	0.35	47	11	11	57
211,251-000	>100	91	0	37	
211,253-000	>100	92	0	27	
211,255-000	>100	94	0	7	
211,257-000	>100	88	10	39	

The invention also relates to methods of treatment of the various pathological conditions described above, by administering to a patient a therapeutically effective amount of the compositions of the present invention. The term administration as used herein encompasses oral, parenteral, intravenous, intramuscular, subcutaneous, transdermal, intrathecal, rectal and intranasal administration.

Binding studies show that certain embodiments of the present invention block NMDA receptors in a stereospecific manner, and that the interaction occurs at binding site(s) distinct from those of some other non-competitive NMDA antagonists or of glutamate and glycine. This, and the other compounds according to formula (I), may therefore prove useful as non-psychoactive drugs that protect against NMDA-receptor-mediated neurotoxicity.

Test systems

Evaluation of the therapeutic effects of the novel PRS-211 compounds has now been carried out in a series of experimental systems to support the utility of these drugs as neuroprotectants, anti-inflammatory agents and anti-oxidative agents. These effects have been evaluated both *in vitro* and *in vivo*, and have been corroborated utilizing the following systems:

(a) Binding to the NMDA receptor linked channel: Non competitive antagonists of NMDA, the primary example of which is the compound MK-801, bind to a site within the NMDA receptor channel thus preventing the activation of the receptor-channel complex and the consequent neurotoxicity. The ability of various compounds to compete with the binding of tritium labeled MK-801 to brain membranes is considered a measure of their potency as NMDA non-competitive antagonists.

(b) Inhibition of prostaglandin production:

The inhibitory effect of the new derivatives of Dexanabinol on prostaglandin synthesis is evaluated in macrophage cell cultures following LPS exposure to induce the inflammatory response. These assays are an indication of the anti-inflammatory activity of the individual analogs.

(c) Inhibition of Tumor Necrosis Factor Alpha:

Specific aspects of the inflammatory response cascade are mediated by the cytokine TNFα. Inhibition of TNFα production and/or inhibition of TNFα release by the new analogs are assayed in macrophage cell cultures activated with LPS. This serves as another indication of the general anti-inflammatory potential of the novel compounds.

(d) Nitric Oxide Assay:

As one aspect of the anti-oxidative potential of the novel analogs they were tested for their ability to inhibit the enzyme nitric oxide synthase (NOS). This assay can also serve as another indication of the anti-inflammatory cascade, as well as the anti-oxidative mechanisms.

(e) Ear Edema model:

The anti-inflammatory activity of the new analogs is screened using an ear edema model in mice. This test system utilizes Croton oil or Arachidonic acid as inflammation inducers. The ability of the test compounds to prevent or diminish the inflammatory response to these stimulants is indicative of their systemic anti-inflammatory capability.

(f) Improved clinical outcome after closed head injury in rats:

Severe head injury is associated with high mortality and severe neurological impairment. Animals subjected to head trauma in a controlled fashion serve as models in which to test drugs of therapeutic potential. Test compounds can be evaluated both for improved clinical outcome and for reduction of edema induced by closed head

injury. The ability of compounds to reduce the severity of neurological symptoms and to reduce brain edema is considered a measure of their potency in reducing brain damage.

(g) Transient Middle cerebral artery occlusion (MCAo): The middle cerebral artery is the cerebral blood vessel most susceptible to stroke in humans. In animals, coagulation, permanent ligation or permanent placement of an occluding thread in the artery produces a permanent focal stroke affecting the MCA territory. Transient ligation or occlusion results in transient focal stroke. Both transient and permanent focal strokes result in varying degrees of edema and infarction in the affected brain regions. The ability of compounds to reduce the volumes of edema and infarction is considered a measure of their potential as anti-stroke treatment.

(h) Optic Nerve Crush: Application of mechanical pressure to the rat optical nerve results in crush injury of the axons, which is accompanied by immediate changes in oxidative metabolism and delayed axonal death and blindness. The ability of compounds to protect the axons and promote axonal sprouting is determined in this assay by following a specific protein for nerve growth cones known as GAP-43.

(i) Parkinson's Disease: The chemical, 1,2,3,6-Tetrahydro-1-methyl-4-phenylpyridine, known as MPTP, is known to cause degeneration of dopaminergic neurons of the substantia nigra, thus providing a model of Parkinson's Disease (PD). The MPTP-induced model in mice is used to evaluate the value of the novel PRS-211 compounds as therapeutic agents for PD.

(j) Myocardial Protection: A rat model of ischemia and reperfusion was used to test the ability of compounds to reduce the volumes of infarction is considered a measure of their potential as cardioprotectors.

Each of these systems represents an aspect of neuroinflammation, neurotoxicity or ischemia, which is amenable to intervention by pharmaceutical agents. The compounds of the present invention exert their demonstrated neuroprotective and anti-inflammatory effects by virtue of a plurality of mechanisms. Certain embodiments of the present invention exert their effect by binding to the NMDA receptor. Among the compounds of the present invention, the amine derivatives in particular have been shown to possess little or no NMDA receptor blocking activity and appear to exert their effect via the cyclooxygenase, lipoxygenase or oxidative pathways. Nevertheless, it

cannot be ruled out that their activity is mediated by other receptors or additional mechanisms.

This evaluation clearly supports the concept that PRS-211 compounds are not acting solely as NMDA receptor antagonists. Rather the therapeutic effects of PRS-211 compounds may be attributable to additional mechanisms including inhibition of tumor necrosis factor, antioxidant and radical scavenger properties, anticholinergic action, platelet activating factor antagonism, anti-inflammatory activity by direct or indirect modulation of arachidonic acid, or inhibition of lipid peroxidation, among others. All of these types of pharmacologic agents have been suggested potentially to improve functional outcome after brain injury. All of these mechanisms may be involved in delayed, secondary or chronic neuronal damage following injury to the CNS (McIntosh, *J. Neurotrauma* 20:215-243, 1993).

The prototype drug used for evaluation of NMDA blocking activity is the compound MK-801, which is a potent and selective NMDA receptor antagonist that cannot be used as a human therapeutic agent due to its toxicity. We have evaluated the similarities and differences between the biological activities of MK-801 and the novel PRS-211 compounds.

Compounds

The currently preferred compounds according to the present invention are novel analogs of the lead compound dexanabinol, also denoted as HU-211, which is disclosed in US Patent 4,876,276. The neuroprotective effects of dexanabinol are disclosed in US Patent 5,284,867.

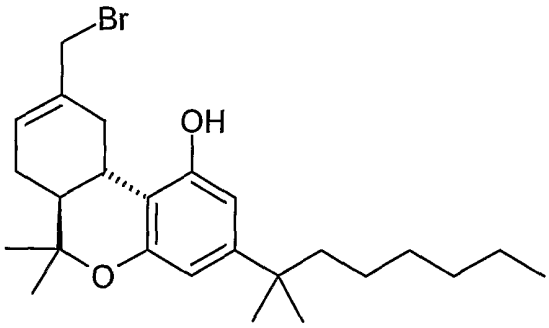
Among the novel compounds tested, analogs of dexanabinol bearing a heterocyclic moiety attached via a methylene bridge at position 1 are currently more preferred. Some of these novel compounds, particularly those having shortened tails compared to dexanabinol, as residue R_2, have the added advantage of being more soluble in some aqueous solutions, whereas the parent compounds are extremely hydrophobic.

These preferred compounds according to the present invention can conveniently be synthesized using dexanabinol as a starting material according to Scheme I, as presented in Figure 1. Alternative synthetic pathways may also be utilized.

The following examples are intended to illustrate the present invention and these are to be construed in a non-limitative manner.

SYNTHETIC EXAMPLES

Synthesis of (+)-(3S,4S)-6,6-Dimethyl-(1,1-dimethylheptyl)-1-hydroxy-9-(bromomethyl)-6a,7,10,10a- tetrahydro-6H- dibenzo[b,d]pyran (Compound 1)



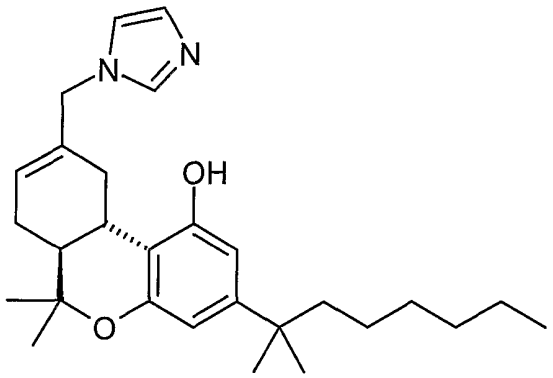
1

Procedure

A suspension of Dexanabinol (1.8 g, 4.66 mmol) and Triphenylphosphine (1.63 g, 6.2 mmol) in acetonitrile (8 ml) was stirred under nitrogen atmosphere at (-10) – (-5) °C. A solution of carbon tetrabromide (2.05 g, 6.2 mmol) in acetonitrile (8 ml) was added in portions. After 1h at ~ -10 °C the reaction was allowed to warm to room temperature. Stirring at 18 °C is continued then for 48 hrs. The solvent was evaporated under reduced pressure at 30°C. The residue was diluted with toluene (10 ml) and the obtained solution filtered through a silica

gel column (40 g suspended in toluene), using toluene as eluent. 1.9 g (y=91%) was collected. Purity was determined by MS, and 1H-NMR.

Synthesis of (+)-(3S,4S)-6,6-Dimethyl-(1,1-dimethylheptyl)-1-hydroxy-9-(imidazolomethyl)-6a,7,10,10a- tetrahydro-6H- dibenzo[b,d]pyran (Compound 2)

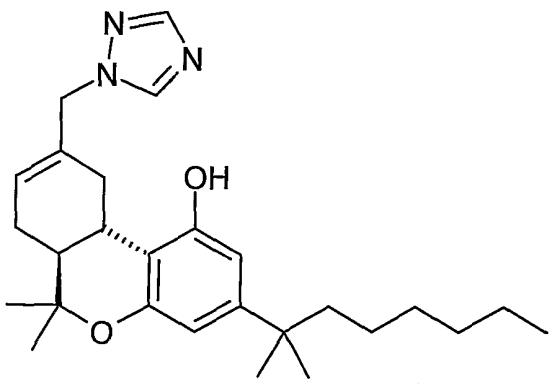


2

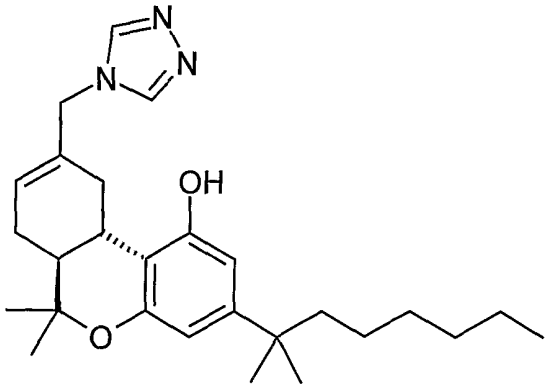
Procedure

A mixture of Compound 1 (100 mg, 0-22 mmole), Imidazole (150 mg, 2.2 mmole) and xylenes (2.5 ml) was concentrated under reduced pressure to a volume of about ~ 1 ml. The mixture was dissolved in anhydrous THF (4 ml) and the solution stirred at (-)10 °C, under nitrogen atmosphere. Butyl lithium (0.9 ml, 18 mmole) was added portionwise. The reaction mixture was allowed to warm slowly to room temperature and stirred for 2.5 hours. The reaction was kept overnight at –20 °C. The reaction was poured into crushed ice (~ 4 g), neutralized with acetic acid and extracted with ether (3 x 20 ml). The organic phase was washed with water (20 ml), dried over $MgSO_4$ and the solvent evaporated. The product was purified by column chromatography on silica gel (5 g), using ethyl-acetate as eluent. 58 mg (yield=60%) of compound 2 was collected and purity determined by MS, and 1H-NMR.

Synthesis of (+)-(3S,4S)-6,6-Dimethyl-(1,1-dimethylheptyl)-1-hydroxy-9-(triazolomethyl)-6a,7,10,10a- tetrahydro-6H- dibenzo[b,d]pyran (Compounds 3a & 3b).



3a



3b

Procedure

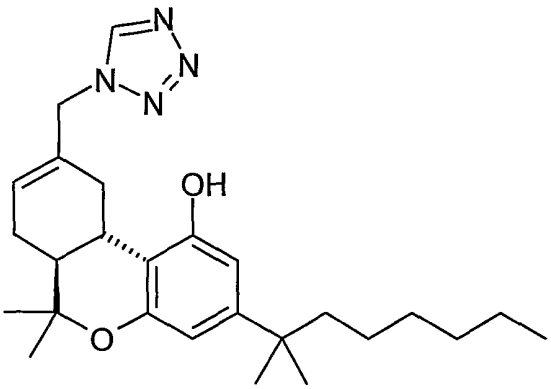
A mixture of Compound 1 (100 mg, 0.22 mmole) and 1, 2, 4 triazole was stirred under nitrogen atmosphere at ~(-5°C). DBU (0.26 ml, 1.78 mmol) was added and the reaction mixture left at room temperature overnight. The reaction was poured onto ice, neutralized with acetic acid, and extracted with ethyl-ether (3 x 10 ml). The organic layer was dried over $MgSO_4$. On TLC two main compounds were seen and were separated by MPLC:

Compound 3a – elution with ethyl-acetate – 47 mg

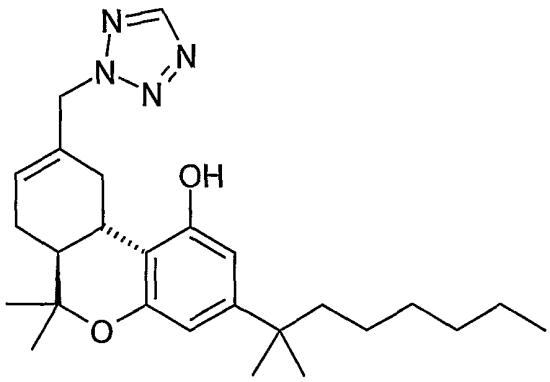
Compound 3b – elution with methanol-ethyl acetate (10 : 90) – 10 mg.

Purity was determined by MS, and 1H-NMR.

Synthesis of (+)-(S,S)-6,6-Dimethyl-(1,1-dimethylheptyl)-1-hydroxy-9-(tetrazolomethyl)-6a,7,10,10a- tetrahydro-6H- dibenzo[b,d]pyran (Compounds 4a & 4b).



4a



4b

Procedure

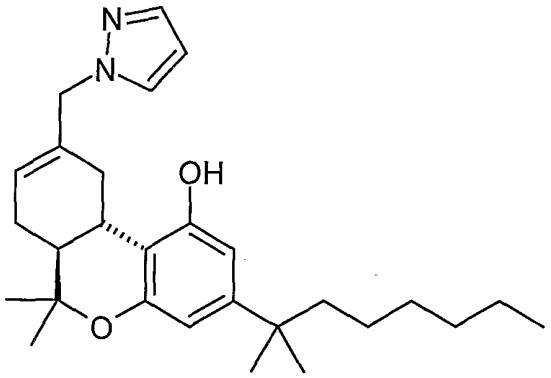
A mixture of Compound 1 (0.25 mg, 0.56 mmole) and tetrazole in dry THF (20 ml) was stirred under nitrogen atmosphere at ~ (-) 5 °C (methanol / ice bath). DBU (0.65 ml, 4.4 mmole) was added (in portions), the reaction mixture was allowed to warm slowly to room temperature, left overnight and left for another 24 hours at 40 ° C to fully dissolve starting material.

The reaction was poured onto ice, neutralized with acetic acid and extracted with ethyl-ether (3 x 30 ml). The organic phase was dried over $MgSO_4$ and the solvent evaporated. On TLC, two main compounds were seen, that could be separated by column chromatography on silica gel (80 gr): Compound 4a (elution with 3% ethyl-acetate in toluene) – 110 mg. Compound 4b (elution with 15% ethyl-acetate in toluene)– 120 mg. The compounds were washed twice with saturated solution of $NaHCO_3$, then with water, dried over Na_2SO_4 and evaporated.

Compound 4a – 64 mg. Compound 4b – 65 mg

Purity was determined by MS, and 1H-NMR.

Synthesis of (+)-(3S,4S)-6,6-Dimethyl-(1,1-dimethylheptyl)-1-hydroxy-9-(pyrazolomethyl)-6a,7,10,10a- tetrahydro-6H- dibenzo[b,d]pyran (Compound 5).

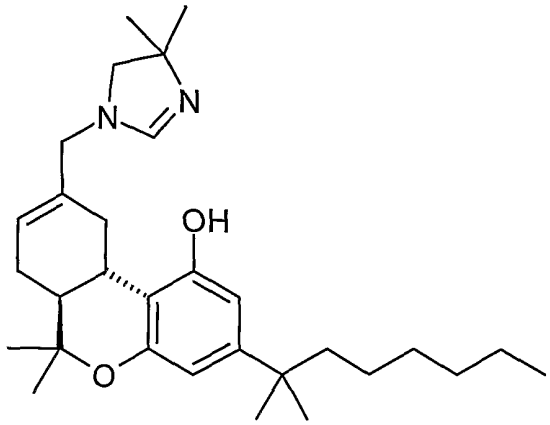


5

Procedure

A mixture of Compound 1 (1g, 2.2 mmole) and pyrazole (1.50 g, 22 mmol) in anhydrous THF (20 ml) was stirred under nitrogen atmosphere while being cooled. N-Butyl lithium (7 ml, 11 mmol) was added portion wise, and the reaction mixture stirred overnight at room temperature. The reaction mixture was poured into crushed ice (~ 4 g), neutralized with acetic acid and extracted with ethyl acetate (3 x 20 ml). The organic phase was washed with water (20 ml), dried over $MgSO_4$ and the solvent evaporated. The product was purified by column chromatography on silica gel (5 g), using 10% ethyl-acetate in petroleum ether as eluent to afford 470 mg (yield=60%) of compound Purity was determined by MS, and 1H-NMR.

Synthesis of (+)-(3S,4S)-6,6-Dimethyl-(1,1-dimethylheptyl)-1-hydroxy-9-(4,4-Dimethyl-2-imidazolomethyl)-6a,7,10,10a- tetrahydro-6H- dibenzo[b,d]pyran (Compound 6)

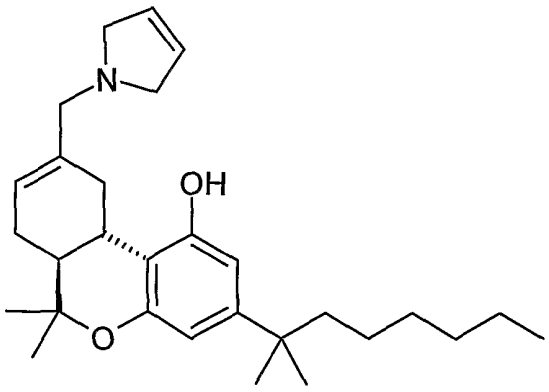


6

Procedure

To a solution of Compound 1 (50 mg, 0.4 mmol) in THF, 4,4-dimethyl-2-imidazoline (0.4 ml) was added and the mixture stirred overnight at room temperature. The reaction mixture was poured onto crushed ice, acidified with acetic acid (pH 5), and extracted with EtOAc. The combined organic phase was dissolved in acetonitrile and water and lyophilized. Yield: 150 mg

Synthesis of (+)-(3S,4S)-6,6-Dimethyl-(1,1-dimethylheptyl)-1-hydroxy-9-(pyrrolinomethyl)-6a,7,10,10a- tetrahydro-6H- dibenzo[b,d]pyran (Compound 7)

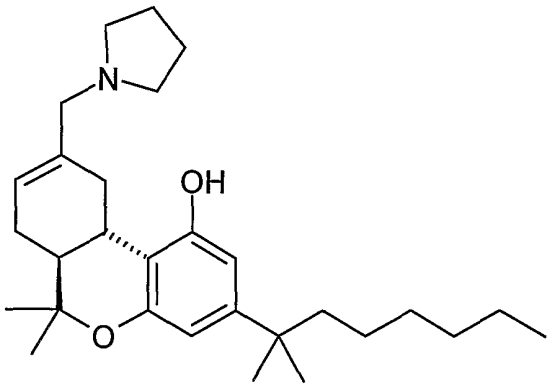


7

Procedure

To a solution of Compound 1 (200 mg, 0.45 mmol) in anhydrous THF (5 ml), pyrroline (0.3 ml) was injected and the mixture stirred at room temperature overnight. The reaction was carried out under nitrogen atmosphere. The mixture was poured into ice, acidified with acetic acid (pH 5) and extracted with EtOAc (3x 20 ml). The combined organic phases were washed with water, dried and evaporated. After lyophilization, 120 mg of product was collected.

Synthesis of (+)-(S,S)-6,6-Dimethyl-(1,1-dimethylheptyl)-1-hydroxy-9-(pyrrolidinomethyl)-6a,7,10,10a- tetrahydro-6H- dibenzo[b,d]pyran (Compound 8)

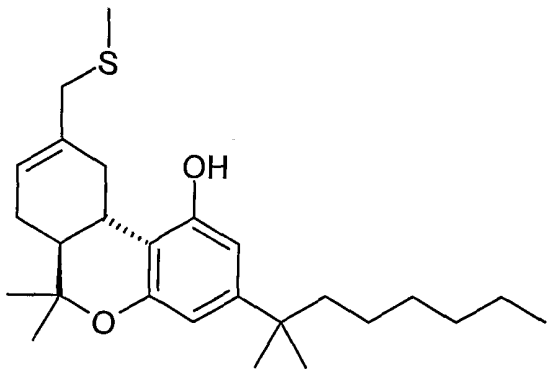


8

Procedure

A mixture of Compound 1 (0.5 g, 1.1 mmol) and pyrrolidine (0.5 ml) in dry THF was stirred at room temperature overnight. The reaction mixture was poured into water, acidified with acetic acid (pH 6) and extracted with EtOAc (3 x 30 ml). The combined organic phase was washed with water, dried and evaporated. The residue was chromatographed over a silica gel column and eluted with 15% methanol in EtOAc. The product obtained was lyophilized to afford 270 mg of pure product.

Synthesis of (+)-(3S,4S)-6,6-Dimethyl-(1,1-dimethylheptyl)-1-hydroxy-9-(methylsulfidomethyl)-6a,7,10,10a- tetrahydro-6H- dibenzo[b,d]pyran (Compound 9)

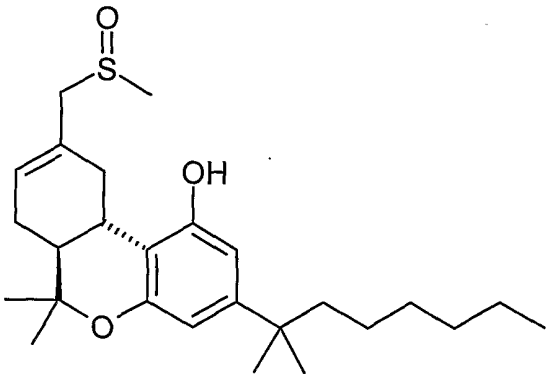


9

Procedure

To a solution of Compound 1 (100 mg, 0.2 mmol) in THF, sodium methyl sulfide was added. The reaction mixture was stirred overnight, evaporated and chromatographed over silica gel to afford 100 mg of compound 9.

Synthesis of (+)-(3S,4S)-6,6-Dimethyl-(1,1-dimethylheptyl)-1-hydroxy-9-(methylsulfoxidomethyl)-6a,7,10,10a- tetrahydro-6H- dibenzo[b,d]pyran (Compound 10)



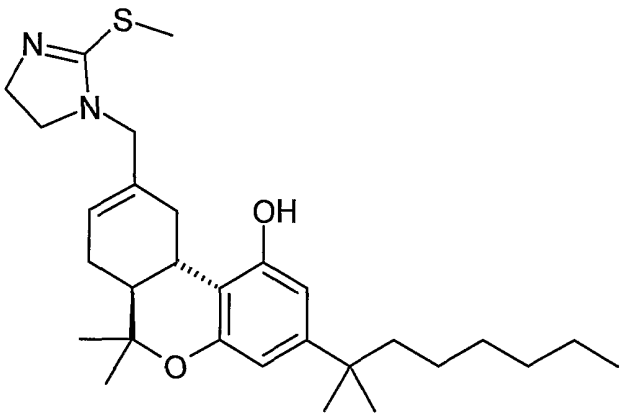
10

Procedure

To a cooled solution (-20° C) of Compound 1 (1.7g, 4 mmol) in dry dichloromethane (120 ml) meta chloroperbenzoic acid (0.83g, 4.8 mmol) was added and the mixture stirred 30 min at the above temperature. The reaction was then poured into a 7%

sodium hydrogenocarbonate solution (250 ml) containing excess sulfite. The product was extracted in dichloromethane (3 x 100 ml), washed with water (2 x 150 ml) dried and evaporated. The residue was chromatographed on silica gel column using 5% EtOH in EtOAc. The product obtained was lyophilized to obtain 1.2g of white powder.

Synthesis of (+)-(S,S)-6,6-Dimethyl-(1,1-dimethylheptyl)-1-hydroxy-9-(2-methylthio-2-imidazolinomethyl)-6a,7,10,10a- tetrahydro-6H- dibenzo[b,d]pyran (Compound 11)

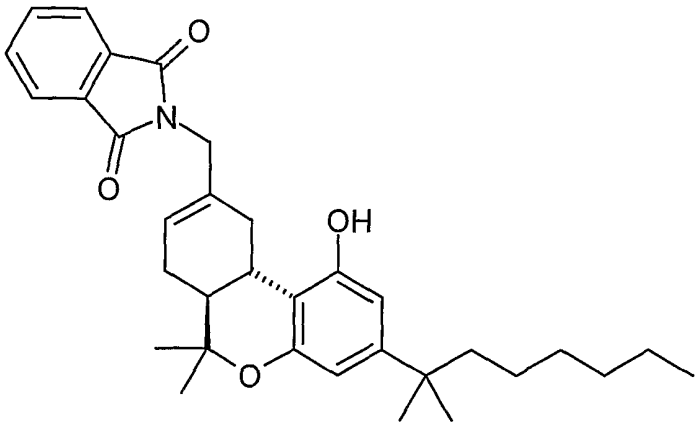


11

Procedure

To a mixture of 2-methylthio-2-imidazoline hydriodide (0.3g, 1.2 mmol) in anhydrous THF (5 ml) DBU (0.18 ml, 1.22 mmol) was added and the mixture stirred at room temperature under nitrogen atmosphere. A solution of Compound 1 (0.2g, 0.4 mmol) in anhydrous THF (2 ml) was injected and the mixture stirred overnight at room temperature. The reaction mixture was poured into water, acidified with acetic acid (pH 5) and extracted with EtOAc. The organic phase was washed with water, dried, with anhydrous sodium sulfate, and evaporated. The residue was chromatographed over silica gel using 25% methanol in ethyl acetate as the eluting system. 120 mg of compound are obtained.

Synthesis of (+)-(S,S)-6,6-Dimethyl-(1,1-dimethylheptyl)-1-hydroxy-9-(phtalimidomethyl)-6a,7,10,10a- tetrahydro-6H- dibenzo[b,d]pyran (Compound 12)

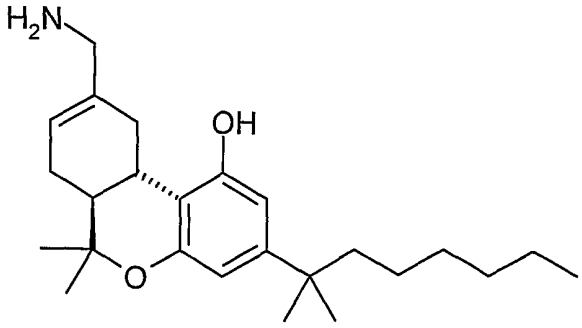


12

Procedure

A mixture of compound 1 (1.2g, 2.15 mmol), phtalimide potassium salt (1.85 g) in methyl sulfoxide (6.0 ml) was stirred under argon at 50 C for 18 hours. The resulting mixture was poured onto crushed ice (20 g), acidified to pH 6 with acetic acid, and extracted with chloroform (2x 20 ml). The extract was dried over sodium carbonate and concentrated under reduced pressure. The residue was subjected to column chromatography using toluene and toluene /ethyl acetate as the eluent to afford 0.4 g of product.

Synthesis of (+)-(3S,4S)-6,6-Dimethyl-(1,1-dimethylheptyl)-1-hydroxy-9-(amino methyl)-6a,7,10,10a- tetrahydro-6H- dibenzo[b,d]pyran (Compound 13)

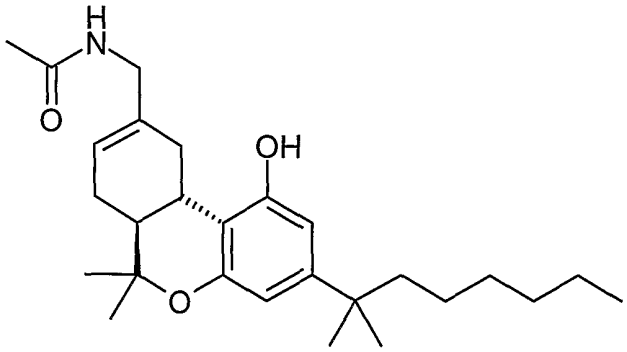


13

Procedure

A solution of Compound 12 (0.516g, 1 mmol) and hydrazine monohydrate (0.159 ml, 3.1 mmol) in ethanol (15 ml) was heated to reflux under argon for 2 hours. Water (0.5 ml) containing concentrated HCl (0.5 ml, 6 mmol) was added and reflux was continued for an additional hour. The solution was left overnight at 22 C. The mixture was diluted with toluene (10 ml). The precipitate was filtered off and washed with ethanol (5 ml). The filtrate and the washings were combined, diluted with ethyl ether (10 ml) and washed with 5% sodium hydrogenocarbonate. The organic phase was separated, dried over sodium carbonate and the solvents were evaporated under reduced pressure. The residue was crystallized from acetonitrile to afford 0.3 g.

Synthesis of (+)-(3S,4S)-6,6-Dimethyl-(1,1-dimethylheptyl)-1-hydroxy-9-(acetamidomethyl)-6a,7,10,10a- tetrahydro-6H- dibenzo[b,d]pyran (Compound 14)

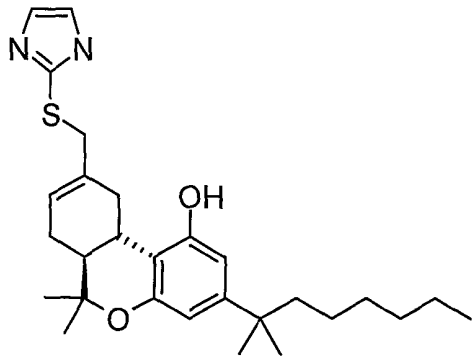


14

Procedure

Compound 13 (0.15 g, 0.39 mmol) was dissolved in acetic anhydride at room temperature. After about 3 minutes a precipitate formed. The mixture was set aside at 22 C for one hour. Methanol (5 ml) was added and all precipitate dissolved giving a transparent solution. The solution was stored at 22 C for 18 hours and the solvent evaporated. The residue was dried in a vacuum oven to give 0.16 g of solid, which crystallized from acetonitrile to afford 0.1g of crystals.

Synthesis of (+)-(3S,4S)-6,6-Dimethyl-(1,1-dimethylheptyl)-1-hydroxy-9-(1H-imidazol-2-ylsulfanyl methyl)-6a,7,10,10a- tetrahydro-6H- dibenzo[b,d]pyran (Compound 15)

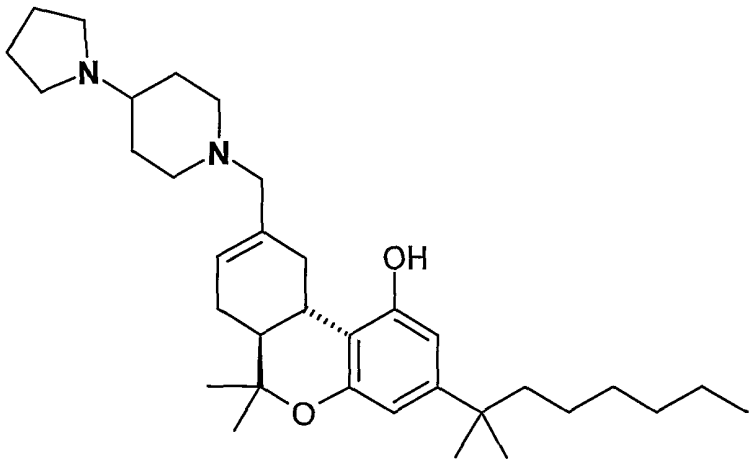


15

Procedure

A mixture of the Compound 1 (2.5gr, 5.6mmole) and 2-mercaptoimidazole (2.2gr, 22mmole) was stirred under nitrogen atmosphere. Triethylamine was added slowly and the obtained mixture was stirred overnight at room temperature. The reaction mixture was poured into water, neutralized with acetic acid and extracted several times with ethyl acetate. The combined organic phase was washed with water, dried over $MgSO_4$ and evaporated to give the crude product which was further purified by silica gel column (elution with 25% ethyl-acetate in petroleum-ether). After lyophilization, compound **15** was obtained as a white powder (yield 1.87gr, 71%):

Synthesis of (+)-(S,S)-6,6-Dimethyl-(1,1-dimethylheptyl)-1-hydroxy-9-(4-(1-Pyrrolidinyl)-piperidinemethyl)-6a,7,10,10a- tetrahydro-6H- dibenzo[b,d]pyran (Compound 16)

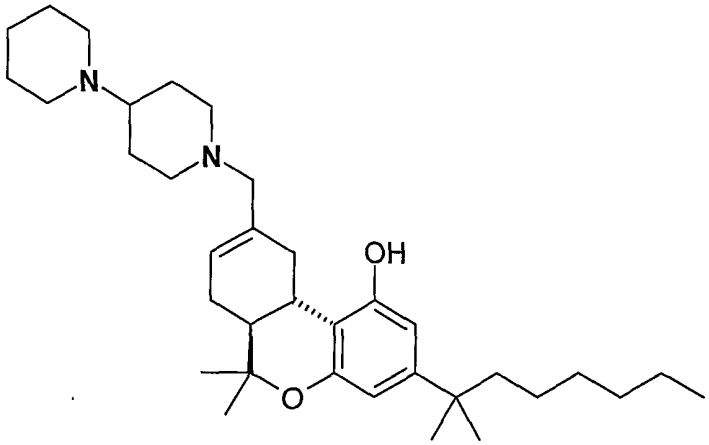


16

Procedure

A solution of Compound 1 (0.3 g, 0.6 mmol) and 4-(1-Pyrrolidinyl)-piperidine (0.2 g, 1.2 mmol) in dry THF (50 ml) was stirred overnight at room temperature. The reaction mixture was diluted with water (30 ml), then acidified with acetic acid and extracted with ethyl acetate (3 x 50 ml). The combined organic fractions were washed with water, dried with sodium sulfate and evaporated to dryness. The desired product was purified by silica gel column chromatography using ethyl acetate-petroleum ether mixture to afford pure product (yield: 85%).

Synthesis of (+)-(3S,4S)-6,6-Dimethyl-(1,1-dimethylheptyl)-1-hydroxy-9-(4-piperidinopiperidine methyl)-6a,7,10,10a- tetrahydro-6H- dibenzo[b,d]pyran (Compound 17).

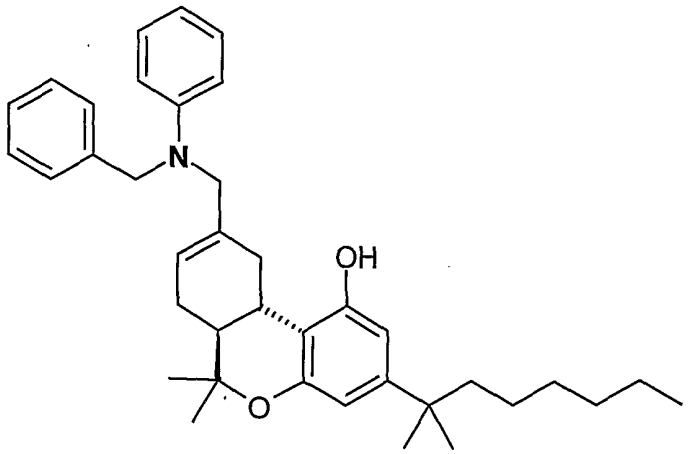


17

Procedure

A solution of Compound 1 (0.3 g, 0. 6 mmol) and 4-piperidinepiperidine (0.21 g, 1.3 mmol) in dry THF (50 ml) was stirred overnight at room temperature. The reaction mixture was diluted with water (100 ml), then acidified with acetic acid and extracted with ethyl acetate (3 x 50 ml). The organic phase was washed with water (50 ml), dried with sodium sulfate and evaporated to dryness. The desired product was purified by silica gel column chromatography using ethyl acetate-petroleum ether mixture to afford pure product (yield 78%).

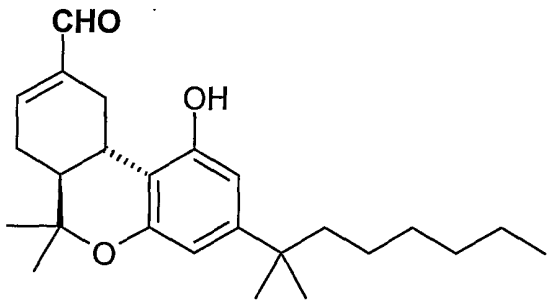
Synthesis of (+)-(S,S)-6,6-Dimethyl-(1,1-dimethylheptyl)-1-hydroxy-9-(N-benzylaniline methyl)-6a,7,10,10a- tetrahydro-6H- dibenzo[b,d]pyran (Compound 18)



18

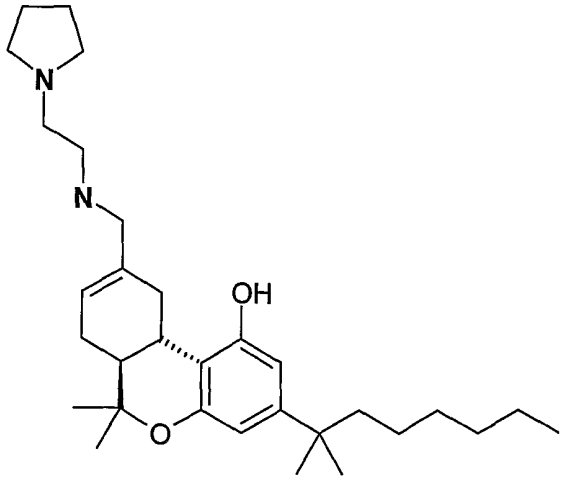
Procedure

A solution of Compound 1 (0.3 g, 0.6 mmol) and N-Benzylaniline (0.23g, 1.3 mmol) in dry THF (50 ml) was stirred overnight at room temperature. The reaction mixture was diluted with water (100 ml), then acidified with acetic acid and extracted with ethyl acetate (3 x 50 ml). The organic phase was washed with water (50 ml), dried with sodium sulfate and evaporated to dryness. The desired product was purified by silica gel column chromatography using ethyl acetate-petroleum ether mixture to afford of pure product (yield: 86%).



Compound 19

Synthesis of (+)-(3S,4S)-6,6-Dimethyl-(1,1-dimethylheptyl)-1-hydroxy-9-(N-(2-Aminoethyl) pyrrolidine methyl)-6a,7,10,10a- tetrahydro-6H- dibenzo[b,d]pyran (Compound 20)

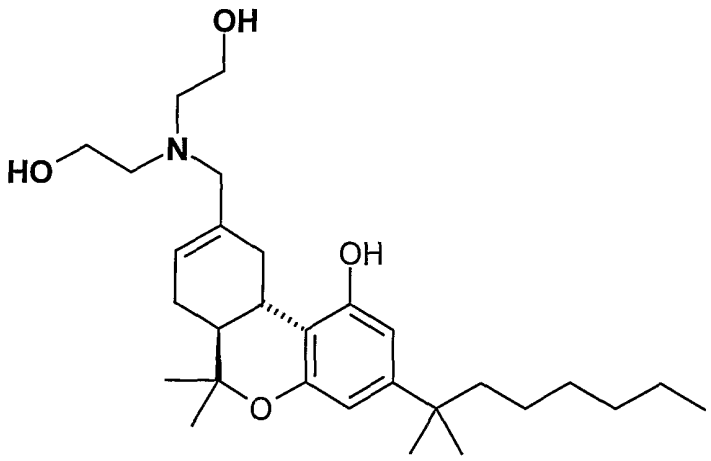


20

Procedure

Compound 19 (0.5 g, 1.3 mmol) and N-(2-Aminoethyl)pyrrolidine (0.44 g, 3.9 mmol) were dissolved in methanol (30 ml) and stirred at room temperature for one hour. Sodium cyanoborohydride (0.72 g) was added and the reaction stirred at room temperature overnight. The reaction mixture was acidified with diluted HCl (1N) and extracted with Ethyl acetate (3 x 50 ml). The organic fraction was washed with water (50 ml), dried over anhydrous sodium sulfate and evaporated under vacuum. The residue was chromatographed over silica gel column using ethyl acetate – petroleum ether as the eluting system to afford the desired product (yield: 69%).

Synthesis of (+)-(3S,4S)-6,6-Dimethyl-(1,1-dimethylheptyl)-1-hydroxy-9-(N-diethanolamine methyl)-6a,7,10,10a- tetrahydro-6H- dibenzo[b,d]pyran (Compound 21)



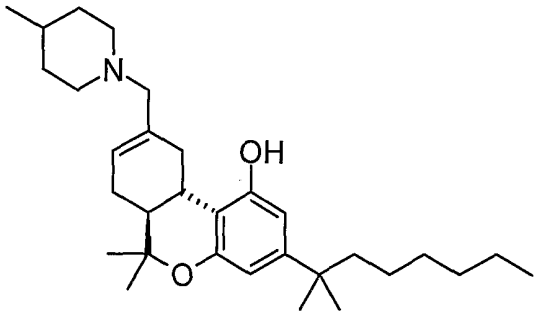
21

Procedure

Compound 19 (0.5 g, 1.3 mmol) and diethanolamine (0.35 g, 3.3 mmol) were dissolved in methanol (30 ml) and stirred at room temperature for one hour. Sodium cyanoborohydride (0.72 g) was added and the reaction stirred at room temperature overnight. The reaction mixture was acidified with diluted HCl (1N) and extracted with Ethyl acetate (3 x 50 ml). The organic fraction was washed with water (50 ml), dried over anhydrous sodium sulfate and evaporated under vacuum. The residue was

chromatographed over silica gel column using ethyl acetate – petroleum ether as the eluting system to afford the desired product (yield: 69%).

Synthesis of (+)-(3S,4S)-6,6-Dimethyl-(1,1-dimethylheptyl)-1-hydroxy-9-(4-methylpiperidine methyl)-6a,7,10,10a- tetrahydro-6H- dibenzo[b,d]pyran (Compound 22)



22

Procedure

4-methyl piperidine (0.47 ml, 6 mmole) was added to a solution of Compound 1 (1.0gr, 2.24 mmol) in dry THF (50 ml) and the obtained mixture was stirred overnight at room temperature. The reaction mixture was poured into water, neutralized with acetic acid and extracted several times with ethyl acetate. The combined organic phases were washed with water, dried over anhydrous $MgSO_4$ and evaporated to give a crude product which was purified by flash silica gel column chromatography (elution with 25% ethyl-acetate in petroleum-ether). After lyophilization, compound 22 was obtained as a white powder (0.62 g).

PHYSIOLOGICAL EXAMPLES

PHYSIOLOGICAL EXAMPLE 1

PRS-211 ANALOGS ANALYZED BY RADIOLIGAND BINDING STUDIES

The identification of possible recognition sites for PRS-211 analogs was carried out by measuring the ability of these PRS-211 analogs to inhibit the binding of MK-801 to rat forebrain membranes. Radioligand binding studies demonstrated that the parent

compound HU-211 competes with the binding of MK-801 to membranes, while it is unable to inhibit AMPA or kainic acid binding.

Forebrain membrane preparation: Brains were removed from Sprague-Dawley rats no more than 5 min after decapitation. Membrane preparations were isolated according to a procedure described previously (Eshhar *et al.*, *Brain Res.* 476:57, 1989). Prior to radioligand binding measurements, endogenous glutamate present in membranes was removed from the preparation by subjecting the membranes to 3-4 successive washings in 10 mM Tris HCl pH 7.2, performed at $4^{o}C$.

Radioligand binding studies: The specific binding of the new analogs to NMDA receptors was determined by their ability to displace (^{3}H)MK-801 from NMDA receptor in rat forebrain preparations. Specifically, rat-forebrain membranes (0.1 mg) were incubated for 2 hours at room temperature with 10 nM of tritiated MK-801 and with the PRS-211 compounds at 3 doses each. Non-specific binding was determined by the use of 0.1 mM MK-801. Following the incubation, bound radioligand was separated from unbound by filtration through GF/B filters. The filters were counted in a β-counter and log of analog concentrations versus % of (^{3}H)MK-801 specific binding was plotted. The IC_{50} was calculated from this plot.

Binding of [^{3}H]MK-801 to membranes was carried out in the presence of 30 μM glycine and 10 μM L-glutamate. Membranes (250 μg protein) were resuspended in 50 mM tris-acetate pH 7.4 buffer and incubated with 10 nM [^{3}H]MK-801, either alone or in the presence of PRS-211 compounds at 0.1-100 μM concentrations for three hours at room temperature (RT). Reaction buffers used in the different radioligand binding studies contained 10% of an ethanol/Emulphor 620/deionized water mixture. The ratio (by volume) of the respective components in the mixture was 20/3/57. This mixture is required for solubilizing PRS-211 compounds at concentrations above 30 μM. Reaction volume was 1 ml. Non-specific [^{3}H]MK-801 binding was determined in the presence of 100 μM unlabeled MK-801.

Concentration dependence of certain PRS-211 analogs on inhibition of [^{3}H]MK-801 binding is illustrated in Figure 1. The inhibition constant (K_I) value displayed by PRS-211,007 was found to be 11.0±1.3 μM. The IC_{50} of several dexanabinol derivatives

is presented in Figure 2. Thus, for example, the mean IC50 of HU-211 (PRS-211,007) was 10 μM compared with that of 2.5 μM for PRS-211,095 (imidazole derivative), versus >20 μM of PRS-211,128 and 4.5 μM for PRS-211,132 and 0.35 μM of PRS-211,220 (pyrazole derivative).

PHYSIOLOGICAL EXAMPLE 2

THE IN VIVO ANTI-INFLAMMATORY EFFECT OF PRS-211 COMPOUNDS IN THE EAR EDEMA MODEL

The anti-inflammatory activity of the new analogs was established in the ear edema model in mice using Croton oil (CO) or Arachidonic acid (AA) as inflammation inducers.

Briefly, the animals were anesthetized and either test analog or identical volume of vehicle is injected IP. CO or AA solution diluted with acetone was injected (ear/ear) to one ear. The contralateral ear, serving as control, received an equal volume of the diluent. One to 3 hours post injection the animals were euthanized and ear thickness measurements are taken in duplicates using a low tension, spring loaded dial micrometer. The edge of the micrometer pads was placed on the outer edge of the ear. Thickness was measured in units of 0.01 mm. Tissue weight was determined by excising a 6 mm diameter disc of ear tissue from the ear lobes using a metal punch. Inflammation/Edema is expressed as the increase in thickness/weight of treated versus diluent treated contralateral ear in animals injected with either test new analogs or vehicle. Dose response curves, with at least 4 doses, are used to calculate potency (ED_{50}), and the maximal efficacy (% of inhibition of Edema), for each of the test drugs.

PHYSIOLOGICAL EXAMPLE 3

IN VITRO SCREENING OF ANTI-INFLAMMATORY ACTIVITY

A. Inhibition of Prostaglandin synthesis

The inhibitory effect of Dexanabinol new analogs on prostaglandin synthesis was evaluated in macrophage cell cultures. Macrophages were seeded in a 24 well NUNC plates, and incubated with DMEM medium for 24 hours to allow attachment to plastic. The wells were vacuumed and a different new analog is added for 1 hour. Treatments are done in triplicates. Following, LPS was added for a duration of 3-24

extra hours, to induce the inflammatory response. The supernatants were collected and analyzed for PGE_2 by enzyme immunoassay technique Biotrak kit (Amersham Pharmacia Biotech). The results obtained with certain preferred novel analogs are summarized in Table 2 above.

B. Inhibition of TNFα

The method for macrophage cell culture growth is identical to the one described in PGE2 assay. Aliquots of the supernatants collected following stimulation with LPS were quantitated for TNFα by enzyme linked immunosorbent assay (ELISA) using HRP conjugated to anti-TNFα antibodies and peroxidase as a substrate for the colorimetric reaction. The peroxidase catalyzed color reaction was stopped by acidification and the absorbance at 450 nm is measured. The absorbance at this wavelength is proportional to the concentration of TNFα in the sample determined from a standard curve plotting the concentrations of TNFα standards versus their absorbance. The results obtained with certain preferred novel analogs are summarized in Table 2 above.

C. Inhibition of Nitric Oxide Synthase (NOS)

The final products of NOS are Nitrite (NO_2) and Nitrate (NO_3). The *in vitro* fluorimetric assay provides an accurate method for the measurement of the Nitrite, which is the majority of NO products. The principle of the fluorimetric assay is based on the addition of DAN (2,3-diaminonaphthalene) to the aliquots of the supernatants collected from macrophage cell cultures incubated with the new analogs and stimulated with LPS. Following, NaOH was added to convert Nitrite to a fluorescent compound 1(H)-naphthtriazole. The fluorescence was measured immediately in a fluorimeter using excitation wavelength of 365 nm and an emission wavelength of 450 nm. The results obtained with certain preferred novel analogs are summarized in Table 2 above.

PHYSIOLOGICAL EXAMPLE 4

THE EFFECT OF PRS-211 COMPOUNDS ON CEREBRAL EDEMA IN A RAT MODEL OF CLOSED HEAD INJURY

The cerebroprotective effect of PRS-211 compounds was assessed in a model of head trauma (HT) in rats. Injury was induced in anesthetized rats by a weight-drop

device followed by a recovery period of up to 48 hours. This type of trauma produces brain edema (i.e. increase in water content, decrease in specific gravity in the brain), breakdown of the blood brain barrier (BBB) and clinical dysfunction. The clinical status of the rats was evaluated 1, 24 and 48 hours after injury along with measuring the extent of cerebral edema. The neurological deficit, assessed by a set of criteria termed the Neurological Severity Score (NSS), is maximal at 1 hour after the initiation of head trauma. The NSS slowly decreases over time from the initiation of HT, with the gradual spontaneous recovery of the rats.

The novel analog PRS-211-095 significantly reduces edema formation and BBB disruption when given before (30 min), immediately after HT (0 min) or even 1 and 2 hours after HT.

The doses required for significant neuroprotection depend on the mode of administration and range from 0.5-20 mg/kg. It is also important to note that the NSS, mainly specific motor function (e.g. beam-walk and balance) improved significantly upon administration of PRS-211. In fact, even one dose of 5 mg/kg of PRS-211-095, given 1 hour after the impact, effectively reduced edema and improved the clinical outcome measured 24 hours after HT.

Experimental procedure:

The model was described in detail by Shapira *et al., Crit. Care Med.* 16:258-265, 1988. Rats were subjected to head trauma (HT) by a weight-drop device and surviving rats were followed up after one week. During that period they had free access to food and water, and were kept 2-3 rats to a cage. At any predesignated time (15 min, 1, 4, 24, 48 hrs, etc.) rats were sacrificed. Their brains were then rapidly removed and cortical tissue taken to determine water content, ions and the metabolites of interest at any particular metabolic cascade studied. During the recovery period, the clinical status was evaluated by a set of criteria (NSS).

Trauma induced a significant decrease in specific gravity (SG) of brain tissue and increase in water content following head injury. Edema developed since more water accumulates in either the extracellular (vasogenic) or intracellular (cytotoxic) spaces. The methods employed to determine edema are based on linear gradient columns of bromobenzene and kerosene (for SG) and for water content on drying the tissue in a desiccated oven. Tissue pieces (20 mg each) were placed on top of the

column and the SG calculated from the equilibrium position in the column, using a standard curve.

Results

Table 3 summarizes the results of a typical experiment in which the novel analog PRS-211-095 was injected at doses of 5-10 mg/kg. The drug was given half an hour before, or one hour after, the induction of trauma and its effect on edema and clinical outcome was evaluated 1 and 24 hours later. The results indicates a significant ($p=0.003$) decrease in the degree of edema developed after head trauma (CHI), as well as a highly significant decrease ($p<0.001$) in the neurological deficit score as a result of PRS-211-095 treatment to traumatized rats.

Table 3. Cerebroprotective effects of HU-211 and PRS-211,095 in rats following Closed Head Injury (CHI)

Treatment	Water content		Neurological score		ΔNSS	N = no. of rats
	Left	Right	1hr	24hr	24hr	
Untreated CHI-control	84.82 ±0.33	79.44 ±0.32	12.2 ±0.6	8.7 ±0.6	3.5 ±0.31	13
Dexanabinol HU-211	83.09 ±0.51 *p=0.007	79.37 ±0.30	11.7 ±0.7	6.3 ±0.6	5.5 ±0.42	8
PRS-211,095	82.89 ±0.48 *p=0.003	79.75 ±0.20	11.9 ±0.6	5.4 ±0.5	6.5 ±0.45	10

The effect of PRS-211 analogs was calculated by the percent edema formation, where 100% was taken as edema in control, non-treated rats. Thus, the reduction in the SG was calculated as follows:

SG (sham) - SG (drug) / SG (sham) - SG (cont) x 100

The increase in water content was calculated as follows:

[%H_20 (drug - % H_20 (sham) / %H_20 (cont) - H_20 (sham)] x 100

All results presented in the table are statistically different ($p<0.05$) from control, traumatized vehicle treated rats.

After the effect on edema was established, when given 30 minutes prior to, or right after, HT, we investigated the "therapeutic window," namely PRS-211, 25 mg/kg i.p. was given one, two or three hours after HT. Its effect on NSS (and on specific motor function) was assessed, as well as the effect on edema and BBB integrity. Figures 6-8 summarize the results of these studies. As can be seen, PRS-211 was fully effective, even when administered up to 2 h post-injury; at 3 h post-trauma the effect was less pronounced.

Conclusion

Severe head injury, or cerebral ischemia, is associated with a high mortality rate (exceeding 50%) and poor functional outcome. Despite extensive clinical and experimental research, there are no well-defined therapies for these conditions. There are very few available treatments for brain injury today and the gradual progressive biochemical changes that occur after head trauma can lead to the evolution of permanent neuronal damage. The results clearly demonstrate that the compounds of the instant invention, namely PRS-211 compounds possess cerebroprotective properties in a model of closed head injury.

PHYSIOLOGICAL EXAMPLE 5

NEUROPROTECTION BY PRS-211 COMPOUNDS IN TRANSIENT MIDDLE CEREBRAL ARTERY OCCLUSION (MCAo), INFARCT SIZE EVALUATION

Experimental design. The design was a randomized one, performed in a masked fashion as to whether drug or vehicle was being given, and an attempt was made to generate approximately equal numbers of drug- and vehicle-treated animals.

Materials

a. Male rats (8 /treatment group) 320-380 gr. (Harlan Israel).

b. Halothane (Rhone Poulenc France)

c. Pentobarbitone (Pental Veterinary, CTS Israel).

d. Poly - L - Lysine (Sigma, USA).

e. Silk suture material 3-0 and 4-0.

f. Nylon (Polyamid) suture material 3-0. Four cm pieces were cut and positioned in a solution of 1% Poly - L - Lysine for 1 minute and dried in an oven (60 ° C) for 60 minutes. The tip of each piece was rounded under a flame.

g. Saline (Teva Medical).

h. Blank cremophor ethanol cosolvent (Pharmos).

i. Dexanabinol in cremophor ethanol cosolvent 50 mg/ml. (Both Dexanabinol and its vehicle were diluted in saline prior to drug administration.

j. Analogs PRS-211,092, PRS-211,095, PRS-211,128 PRS-211,132 and PRS-211,220 in cremophor ethanol cosolvent, 50 mg/ml.

k. The analogs were diluted in saline prior to drug administration.

Methods

Transient MCAo

a. Surgical details of the MCAo method used for testing dexanabinol analogs, are presented in Belayev, et al., (Belayev, Busto, Weizhao, and Ginsberg, *Stroke* 26:2313-2319, 1995) which is incorporated herein in its entirety by reference.

b. Two hours after the start of MCAo, the animals were reanesthetized with halothane, the neck wound was re-opened and the nylon thread pulled out of the Internal carotid artery (ICA). The skin wound was then closed with 3-0 silk suture material and the animals allowed to recover from the anesthesia.

c. Three sets of animals were tested. In the first test immediately (2+0 hours) before thread removal the animals were injected IV with either analogs PRS-211,095, PRS-211,128, PRS-211,132, or Dexanabinol (PRS-211,007) each at a dosage of 5 mg/kg. One group of animals was treated with 5ml/kg vehicle alone. In another group, the animals subjected to "sham" operation, the suture was passed into the ICA, as described above, but immediately withdrawn. In the second set dexanabinol, PRS-211,092, PRS-211,095 and PRS-211,220 were used, at 2+0 hours. In the third set dexanabinol, PRS-211,092, PRS-211,095 and PRS-211,220 were administered 1 hour after thread removal (2+1 hours). Laser Doppler Flow (LDF) determined the success of MCAo - a drop of more then 50% in cerebral blood flow was considered as a sign of successful MCAo. Although LDF was a helpful corroborative sign, clinical outcome remained the final inclusion/exclusion criterion, since, with the exception of the PRS-211,220 staircase study, LDF was introduced only after initiation of the study.

Behavioral / neurological outcome assessment

A detailed investigation of neurological performance was carried out on the first, third and the 7th day after the MCAo. Two parameters were examined: posture and the flexion reflex (based on Nokon and Chuang, *NeuroReport* 9, 1998: 2081-2084).

Animals were scored according to their performance:

Posture Score

0 – Normal

1 – Slight twisting

2 – Marked twisting

3 – Marked twisting and forelimb flexion

Flexion Reflex Score

0 – Normal

1 – Slight deficit

2 – Moderate deficit

3 – Severe deficit

Morphological assessment of the infarct size

One week after the ischemic insult, animals were euthanized with pentobarbitone 100 mg/kg IP. The animals were perfused through the heart with heparinized 4% formaldehyde solution in PBS (pH 7.4). Brains were then removed, and kept in the same solution before preparation for histological evaluation of the brain infarct volume.

Statistical Analysis

The infarct size was compared using ANOVA (analysis of variance) followed by Duncan's post hoc test.

Results

a. Mortality rate

No mortality was detected in the sham and 211,095 treated rats. A low mortality rate (9%) was seen in the Dexanabinol treated animals. The mortality rate among the other treatment groups (vehicle, 211,128 and 211,132) was similar (around 25%).

b. Behavioral / Neurological outcome

Animals treated with analog PRS-211,095 demonstrated fewer neurological deficits and recovered faster compared to the other treatment groups.

c. Neuropathology

The means of the infarct volume and the percentage relative size to the contralateral hemisphere were least in animals treated with PRS-211,220 PRS-211,092 and PRS-211,095, the first at 0.5 mg/kg, followed by the values in animals treated with 211,132 (PRS-211,204 gave also protected by 50%). The reduction of infarct size was 60%, 52% and 48% for PRS-211,095, PRS-211,092 and dexanabinol (HU-211, PRS-211,007), respectively.

Conclusions

These results show that the novel analogs PRS-211,095, PRS-211,092 and PRS-211,220 are potent both in terms of preservation of function and reduction of brain lesion size after MCAo. These data can be interpreted in the context of the relatively high affinity of PRS-211,095 and PRS-211,220 for the NMDA receptor as well as being potent inhibitors of COX-2. In contrast, PRS-211,128 has no detectable affinity for the NMDA receptor and was inactive in terms of function, reduction of mortality and brain morphometry. These structure-activity relationships suggest that affinity for the NMDA receptor is an important, but not the only, component for neuroprotection against ischemia. PRS-211,092 shows low affinity for the NMDA receptor and suggests that other, unidentified mechanisms are important for neuroprotection.

PHYSIOLOGICAL EXAMPLE 6

NEUROPROTECTION BY PRS-211 COMPOUNDS IN TRANSIENT MCAo: EVALUATED BY THE STAIRCASE TEST.

The test challenges fine motor, sensory and stereognostic function of the cortex in enabling the forepaw (hand) to identify, grasp and accurately manipulate small objects such as food pellets. Loss of the ability to identify, grasp and manipulate small objects reflects a lesion of the fronto-parietal cortex and is a common and crippling deficit after stroke in humans.

Materials

The materials for this procedure are identical to those from **Physiological Example 5**, except for the different concentrations of PRS-211,095. The procedural differences are as follows:

- Male Sprague Dawley rats, 230-270 gr (Harlan, Israel) were used. The following compounds and their respective concentrations were tested:
Dexanabinol and analogs PRS-211,095 and PRS-211,220 in PEG ethanol cosolvent 50 mg/ml. Dexanabinol, the analogs and their vehicle were diluted in Intralipid® 20% (Pharmacia) prior to drug administration.

Methods

1. Training for functional evaluation

The procedure is essentially as described in Montoya et al. (Montoya, Campbell-Hope, Pemberton and Dunnet, *J. Neurosci. Meth.* 36:219-228, 1991) Animals were kept under mild food deprivation for 3-5 days, receiving 15-gr. food once a day (between 16:00 and 17:00) and free access to water. Animals were trained prior to the test for 3-5 days according to the protocol of Sharkey et. al. (Sharkey, Crawford, Butcher and Martson, *Stroke* 27:2282-2286, 1996).

In brief, the staircase box contains two rows of seven stairs in each. Two 45 mg food pellets were placed on each step. Animals were placed in the box for 15 minutes sessions. At the end of the session the number of eaten (grasped) and displaced pellets was counted and recorded. Animals were tested twice a day, 4 hours apart. The first session was performed between 8-9 AM and second between 12-13 PM. The animals were trained until they grasped (ate) at least 8 pellets from each set of stairs for two consecutive sessions. Up to one week after the end of the successful training session the animals underwent transient MCAo. Transient MCAo was performed in essentially the same manner as described in **Physiological example 5** with the following modification at step i:

Two minutes prior to thread removal rats were re-anesthetized and administered IV with Dexanabinol 5mg/kg, analogs PRS-211,220 or PRS-211,095 at 0.5, 2.5, 5 or 10 mg/kg or vehicle 5 ml/kg. One additional group was sham-treated.

2. Functional assessment following transient focal ischemia

Animals were allowed to recover from the surgery for five days. The mild food deprivation was re-employed during the days following the ischemic insult. They were tested in the staircase box using the above method for 9-12 days. Animals were weighed twice a week during this period.

3. Morphological assessment of the infarct size

At the end of the evaluation period, animals were euthanized with pentobarbitone 100 mg/kg IP. The animals were perfused through the heart with heparinized 4% formaldehyde solution in PBS (pH 7.4). Brains were then removed, and kept in the same solution for at least 24h. Then the brains – from the rostral side of the cortex to the cerebellum- were sunk in 30% sucrose in PBS, cryosectioned (20 □m), dried and stained with thionin for histological evaluation of the brain infarct volume. Eight sections at the levels of: 3.3;+2.8;+1.8; +0.8; -0.4;-1.4; -2.2; and –3.4 from Bregma were measured (Swanson, Rat Brain Atlas, 1992). Sections were captured by a CCD camera (V-tech MP-470) and image analyzed (Scion image 1.62A). Infarct size was determined from the series of the 8 sections by either calculation of the infarct volume (mm3) by summing the series of mean lesion areas of two adjacent sections multiplied by the distance between them or calculation of the mean infarct size as percentage of the contralateral hemisphere size; calculation of the mean of the surviving ipsilateral side as percentage of the contralateral hemisphere size; calculation of the mean ipsilateral side ventricle as percentage of the contralateral hemisphere ventricle.

4. Statistical Analysis

a. The infarct size was compared using ANOVA (analysis of variance) followed by Duncan's post hoc test.

b. The staircase task performance was evaluated after collecting the data and compressing it into four blocks of six trials:

First block– The last day of preoperative testing

Second block–Tests from day 6, 7 and 8 post ischemic insult

Third block- Tests from day 9, 10 and 11 (or 12 or 13) post ischemic insult (a pool of the second 3 days)

Fourth block- Tests from day 14-18 post ischemic insult

c. Data were analyzed using ANOVA (analysis of variance) followed by Tukey's post hoc test.

Results

a. Physiological parameters

No differences in the two measured physiological (blood glucose and rectal temperature) among the different treatment groups or at different treatment times were detected.

b. Mortality Rate

Mortality in the present study was low as can be seen in Tables 4 and 5.

Table 4: Mortality rate for PRS-211,095 treated animals

Treatment	N	No. Dead Animals	Mortality Rate(%)
Sham	13	0	0
Vehicle	13	1	8
Dex 5	8	0	0
211,095 0.5	14	3	21
211,095 2.5	10	3	30
211,095 5	9	0	0
211,095 10	11	1	9

Table 5: Mortality rate for PRS-211,220 treated animals

Treatment	N	No. Dead Animals	Mortality Rate (%)
Sham	18	0	0
Vehicle	25	4	16
211,220 0.1	16	4	25
211,220 0.5	16	3	18.75
211,220 2.5	13	4	30.7
211,220 5	14	2	14
Dex 0.5	11	4	36

c. Body Weight gain

Animals were kept under mild food deprivation during the staircase testing (except during weekends). Under these conditions, the following results were observed:

Sham and PRS-211,095 10 mg/kg treated rats gained the maximal body weight (15 and 17% respectively compared to base line). This was statically different ($p<0.05$) from vehicle-treated rats, which did not gain weight at all. (They even

lost 4% of their body weight) Dexanabinol and PRS-211,095 (both 5 mg/kg) and PRS-211,220 0.1, 0.5, 2.5 and 5 mg/kg treated rats demonstrated a moderate body weight gain (7 and 4% for PRS-211,095 and 3, 10, 5 and 4% for PRS-211,220, respectively). These differences were not statistically different. The other groups (PRS-211,095 0.5 and 2.5 mg/kg) did not gain weight.

d. Staircase Test Performance

No significant differences in the ipsilateral performance were observed among the different treatment groups. The mean number of pellet consumption was between 8-11 per test. There was a slight decrease in pellet consumption detected in all treatment groups during the first session following the ischemic insult (the end of the first week post insult). This decrease was transient and disappeared during the next sessions.

A marked impairment was evident in the vehicle-treated rats for contralateral performance. Pellet consumption was reduced to less than 3 in the first session following the ischemic insult. This went up to 5 pellets in the third session. PRS-211,095 treated rats demonstrated an improved performance in a dose-related manner. PRS-211,095 0.5 and 2.5 mg/kg treated animals showed moderately improved pellet consumption, while the 5 and 10 mg/kg dose levels demonstrated a robust improvement ($p<0.05$ compared to vehicle).

Dexanabinol 5 mg/kg had a similar effect. PRS-211,095 5 mg/kg was superior to the 10-mg/kg dosage and to the Dexanabinol only in session 1. No improvement was detected in the Dexanabinol 0.5 mg/kg treated rats. PRS-211,220 treated rats demonstrated an improved performance in a dose-related manner. PRS-211,220 0.1 2.5 and 5.0 mg/kg treated animals showed moderately improved pellet consumption (20-30% improvement over vehicle), while the 0.5 mg/kg dose level demonstrated a robust improvement (more then 60% relative to that of vehicle alone: $p<0.05$). The best performance in The last session (session no. 3) was seen with PRS-211,220 0.5 mg/kg.

A similar phenomenon was detected in the displaced pellets parameter. The vehicle-treated rats displaced the highest number of pellets, while the sham-operated rats displaced the least. PRS-211,095 and Dexanabinol 5 mg/kg and

PRS-211,220 0.5 mg/kg induced an improved performance similar to that seen in the previous parameter

Figure 11 depicts contralateral performance in the staircase test. A. Results for PRS-211,095 pellets eaten and pellets displaced and B. Results for PRS-211,220 pellets eaten and pellets displaced.

e. Histopathology

7-8 animals in 3 treatment groups underwent histopathological analysis: Dexanabinol 5 mg/kg, PRS-211,095 at 2.5 and 5 mg/kg. Under the treatment conditions, Dexanabinol and PRS-211,095 5 mg/kg reduced the infarct volume by 35% compared to vehicle. This effect was not statistically different. No protection was seen with any other dosage. Figure 12 shows the change in cerebral infarct size in animals treated with certain preferred dexanabinol derivatives as assessed in the transient MCAo test. The left panel shows results of preferred PRS-211 compounds in the 2+0 hour assay, where the preferred compounds were administered immediately upon MCAo completion. The right panel shows results of preferred PRS-211 compounds in the 2+1 hour assay.

Conclusions

First, Analog PRS-211,095 and Dexanabinol demonstrated similar neuroprotection following 120 minutes of transient MCAo. This was evident from the contralateral performance in the staircase as well as from the reduction in infarct size. PRS-211,220 at 0.5 mg/kg had the most potent effect in session 3 of the staircase. This is at least 10 times more potent than PRS-211,095 and dexanabinol. Second, all dose levels of PRS-211,095 demonstrated improved performance in the staircase test, but the best activity was seen with the 5 mg/kg dose. It is worth noting that PRS-211,220 at the 0.5 mg/kg dose performs better than PRS-211,095 or dexanabinol, both at a 5 mg/kg dose.

The histopathological evaluation revealed that both Dexanabinol and analog PRS-211,095 (both at the 5 mg/kg dose) reduced the infarct size by 35% (compared to vehicle).

PHYSIOLOGICAL EXAMPLE 7

NEUROPROTECTION BY DEXANABINOL ANALOGS IN THE MPTP MODEL OF PARKINSON'S DISEASE (PD)

The MPTP-mouse model of Parkinson's disease is used to test the efficacy of the compounds to prevent the onset of PD symptoms.

Materials

a. 190 eight week old male C57/BL mice (Harlan Israel).
b. MPTP-HCl (Sigma USA).
c. Saline (TevaMedic Israel).
d. Rat antibody against MAC-1 or F4/80 (Serotec).
e. Rabbit anti-GFAP (Sigma).
f. Rabbit anti- e,n or iNOS (Serotec)
g. Sheep anti-Tyrosine Hydroxylase (TH) (Calbiochem).
h. Secondary antibodies (Zymed)
i. Pentobarbitone sodium 200 mg/m (CTS Israel).
j. Heparin (500 IU/ml, Chaoi France).
k. 4% formaldehyde buffer solution (Frutarom Israel).

Methods

The protocol is based on Liberatore et al. (Liberatore, Jackson-Lewis , Vukosavic , Mandir, Vila , McAuliffe, Dawson, Dawson and Przedborski, *Nature Medicine* 5:1403-9, 1999).

The treatment groups are as follows:

- Vehicle 5 ml/kg IP once just before MPTP administration.
- Dexanabinol or analog 10 mg/kg IP once just before MPTP administration.
- Dexanabinol or analog 20 mg/kg IP once just before MPTP administration.
- Dexanabinol or analog 20 mg/kg IP once just before MPTP administration + additional dose 24 hours later.

Table 6 depicts the experimental strategy followed to determine the most effective treatment in the MPTP model. 19 groups of animals are treated as follows:

Table 6

Days post MPTP	**TREATMENTS***					
24 hours	MPTP	Saline	MPTP+ Dex (10mg)	MPTP+ Dex (20mg)	MPTP+ Dex (30mg)	MPTP+ Dex (20mgx2)
3 days	MPTP	Saline	MPTP+ Dex (10mg)	MPTP+ Dex (20mg)	MPTP+ Dex (30mg)	MPTP+ Dex (20mgx2)
7 days	MPTP	Saline	MPTP+ Dex (10mg)	MPTP+ Dex (20mg)	MPTP+ Dex (30mg)	MPTP+ Dex (20mgx2)
Naive						

***Dex = dexanabinol or analog**

Seven days after MPTP administration the mice are euthanized and their brains removed cut and stained using an antiserum to tyrosine hydroxylase (TH-IR), the rate limiting enzyme in monoaminergic neurons. Attention is focused on the Substantia nigra, pars compacta (SNpc), which are comprised of dompaminergic neurons that project to the striatum. It is these neurons that undergo degeneration in Parkinson's Disease. MPTP acts as a specific neurotoxin for these neurons, and the aim is to determine whether MPTP-induced nerve degeneration can be ameliorated by the novel PRS-211 compounds.

PHYSIOLOGICAL EXAMPLE 8
OPTIC NERVE CRUSH MODEL

The novel compounds are tested in an Optic Nerve Crush model to determine their effects in axonal survival and regeneration.

Materials

a. Adult, male Sprague Dawley rats 350-550 gr. (Harlan Israel).

b. Dexanabinol or analogs 5% in Cremophor Ethanol (Pharmos).

c. Blank Cremophor Ethanol (Pharmos).

d. Pentobarbitone (Pental Veterinary, CTS, Israel) 1mg/kg, 1:5 with Saline

e. Xylazine (Vitamed) 1mg/kg, 1:5 with Saline

f. Antibodies; Mab anti-GAP43 (Sigma, G-9264), anti-GFAP (Sigma)

Methods

The methodology is as described in Duvdevani et al., (Duvdevani, Rosner, Belkin, Sautter, Sabel, and Schwartz, *Rest. Neurol. Neurosci.* 2:31-38, 1990).

Evaluation

Optic nerves

At the end of the experiment (8 weeks) the animals are deeply anesthetized with pentobarbitone 60 mg/kg IP and cardially perfused with 4% heparinized formaldehyde solution. The eye and the optic nerves from the globe to the chiasm are removed and further fixed by immersion in 4% paraformaldehyde overnight. The optic nerves (from the globe to the chiasm) and the brains (the areas of the lateral geniculate body and the superior colliculus) are cryoprotected in 30% sucrose, frozen and sectioned (serial sections: Optic nerves- longitudinal, 16μm; Brains- coronal, 30μm). Retinas are prepared as whole mounts. The retinas and sections are immunohistochemically stained with anti-GAP-43 and anti-GFAP. GAP-43 is an acidic, axonally transported membrane protein present in the CNS and PNS whose presence is indicative of regenerative growth (Skene and Willard, *J Neurosci* 1:419-26 1981) while GFAP, Glial fibrillary acidic protein, is a marker for astrocytes. GAP-43 positive labeling indicates regenerative growth while GFAP positive labeling is indicative of glial scar formation. Treatments demonstrating positive labeling for GAP-43 are repeated and processed for electron microscopy analysis. The number of viable axons in each treatment, as a measure for neuroprotection and the number of unmyelinated, thinly myelinated axons and growth cones, as a measure of regeneration, are compared in a cross section, 1mm distal from the site of injury. In animals demonstrating regenerative growth, we measure the length of regenerating axons.

PHYSIOLOGICAL EXAMPLE 9
RAT MODEL OF MYOCARDIAL ISCHEMIA

The following model of myocardial infarction and heart failure was used to test the ability of compounds to reduce the volumes of infarction is considered a measure of their potential as cardioprotectors.

Materials and Methods

The methods used are essentially as described in Leor and Kloner (Leor and Klonner, *Am. J Cardiol.* 75:1292-3 1995). The experiment was performed in a masked fashion. In brief, Sprague-Dawley rats were given either vehicle or PRS-211,092 15 minutes before occlusion. They were subjected to 45 minutes of coronary occlusion and 4 hours of reperfusion, after which the coronary artery was reoccluded and 0.25 ml of Unisperse™ dye was injected IV to determine area of risk (AR). The rats were euthanized and hearts were analyzed as to infarct size using a 1% solution of triphenylteuazolium chloride for 15 minutes at 37°C. The area of necrosis (AN) in each heart was expressed as a percentage of the area at risk. This was multiplied by the weight of each slice to obtain the mass of tissue at risk and necrosis.

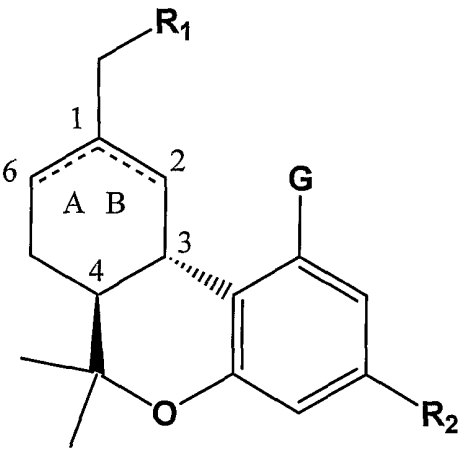
Results and Conclusions

Figure 13 shows the area of necrosis per area of risk (AN/AR) for R (PRS-211-092) and S (vehicle) and the area of necrosis of the left ventricle (AN/LV). The Y axis measures the effect on infarct volume in mm^3. There is a clear decrease in infarct size in animals treated with the novel dexanabinol derivative, PRS-2111-092.

Although the present invention has been described with respect to various specific embodiments thereof in order to illustrate it, such specifically disclosed embodiments should not be considered limiting. Many other specific embodiments will occur to those skilled in the art based upon applicants' disclosure herein, and applicants propose to be bound only by the spirit and scope of their invention as defined in the appended claims.

THE CLAIMS

1. A compound of the general Formula (I):



having the (3S,4S) configuration and being essentially free of the (3R,4R) enantiomer, wherein A-----B indicates an optional 1(2) or 6(1) double bond,

R_1 is

A) R3 where R3 is selected from the group consisting of

a) a linear or branched, saturated or unsaturated, carbon side chain comprising 1-8 carbon atoms interrupted by 1-3 heteroatoms; or

b) a saturated or unsaturated cyclic moiety, an aromatic moiety or a heterocyclic moiety; the cyclic moiety having from 5-20 atoms comprising one or two-ringed structures, wherein each ring comprises 3-8 carbons, interrupted by 1-4 heteroatoms, said heteroatoms each independently selected from the group consisting of N, O, and S; wherein each ring optionally is further substituted with one or more groups selected from

i) C_{1-6} alkyl,

ii) C_{1-6} alkoxy,

iii) C_{1-6} alkylthio,

iv) Halo,

v) Carboxyl

vi) $-CO_2-C_{1-4}$ alkyl

vii) keto,

viii) nitro,

a saturated or unsaturated cyclic moiety, or an aromatic or a heterocyclic moiety as defined in b above;

B) an amine or an amide substituted with at least one substituent as defined in R3 above;

C) a thiol, a sulfide, a sulfoxide, a sulfone, a thioester or a thioamide optionally substituted with one substituent as defined in R3 above;

D) an ether –OR3 wherein R3 is as defined above;

G is (a) halogen, (b) C_1-C_5 alkyl, or (c) -OR wherein R is (a') -R", wherein R" is hydrogen or C_1-C_5 alkyl optionally containing a terminal -OR'" or -OC(O)R'" moiety wherein R'" is hydrogen or C_1-C_5 alkyl, or (b') -C(O)R'" wherein R'" is as previously defined,

and $\mathbf{R_2}$ is (a) C_1-C_{12} alkyl, (b) -OR"", in which R"" is a straight chain or branched C_2-C_9 alkyl which may be substituted at the terminal carbon atom by a phenyl group, or (c) $-(CH_2)_nOR'''$ wherein n is an integer of 1 to 7 and R'" is hydrogen or C_1-C_5 alkyl;

with the proviso that $\mathbf{R_1}$ is other than a heterocyclic moiety having a labile hydrogen atom so that said moiety acts as a carboxylic acid analogue.

2. The compound according to claim 1 wherein $\mathbf{R_1}$ is a saturated or unsaturated cyclic moiety, an aromatic moiety or a heterocyclic moiety having from 5-20 atoms comprising one or two-ringed structures, wherein each ring comprises 3-8 carbons interrupted by 0-4 heteroatoms, said heteroatoms in each ring independently selected from the group consisting of N, O, and S; optionally further substituted with at least one substituent selected from the group consisting of lower alkyl, halogen, nitro,

cyano, -SR''', -NHR''', -N(R''')$_2$, -OR''', -COR''' , -C(O)OR''' or NH-COR''' moiety wherein R''' is hydrogen or C_1-C_5 alkyl.

3. The compound according to claim 1 wherein $\mathbf{R_1}$ is a heterocyclic moiety selected from the group consisting of an imidazolyl, an imidazolinyl, a morpholino, a piperidyl, a piperazinyl, a pyrazolyl, a pyrrolyl, a pyrrolidinyl, a triazolyl, and a tetrazolyl, the heterocyclic moiety optionally further substituted wherein the substituent is selected from the group consisting of C_{1-6} alkyl, C_{1-6} alkyloxy, C_{1-6} alkylthio, keto, carboxy , or nitro.

4. The compound according to claim 1 wherein $\mathbf{R_1}$ is imidazolyl.

5. The compound according to claim 1 wherein $\mathbf{R_1}$ is pyrazolyl.

6. The compound of claim 1 where $\mathbf{R_1}$ is 2-methyl thio-2-imidazolinyl.

7. The compound of claim 1 where $\mathbf{R_1}$ is 4-methylpiperidine.

8. The compound according to claim 1 wherein A-----B is a 6(1) double bond, and G is –OH, or lower acyloxy.

9. The compound according to claim 9 in which and $\mathbf{R_2}$ is 1,1-dimethylheptyl or 1,2-dimethylheptyl.

10. The compound of claim 9 wherein $\mathbf{R_1}$ is selected from the group consisting of imidazole, pyrazole, oxazole, isoxazole, tetrahydropyridine, pyrazoline, oxazoline, pyrrolidine, imidazoline, 2-thio-imidazole, 2-methylthio-imidazoline, 4-methyl-2-imidazoline, 4,4-dimethyl-2-imidazoline, methyl sulfide, methylsulfoxide, acetamido, benzamide, cyano, 1,2,4-triazole, 1,3,4-triazole, 1,2,3,4-tetrazole, 1,2,3,5-tetrazole, thiophene, phenyl, morpholine, thiomorpholine, thiazolidine, glycerol, piperazine, piperidine, 4-methylpiperidine and tetrahydropyran, the heterocyclic moiety

optionally further substituted wherein the substituent is selected from the group consisting of C_{1-6} alkyl, C_{1-6} alkyloxy, C_{1-6} alkylthio, keto, carboxy , or nitro.

11. The compound of claim 10 wherein $\mathbf{R_1}$ is imidazole.

12. The compound of claim 10 wherein $\mathbf{R_1}$ is pyrazole.

13. The compound of claim 10 wherein $\mathbf{R_1}$ is 2-methyl thio-2-imidazoline.

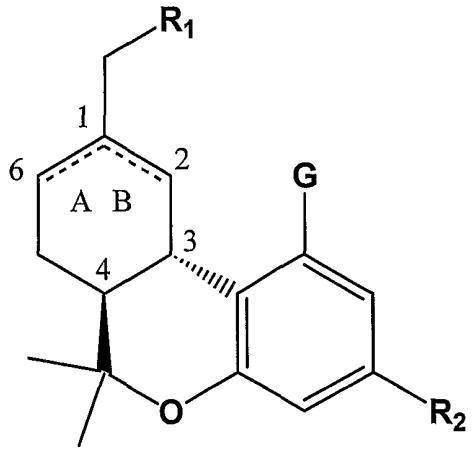
14. The compound of claim 10 wherein $\mathbf{R_1}$ is 4-methylpiperidine

15. The compound according to claim 10 wherein G is –OH, or lower acyloxy group.

16. The compound according to claim 10 wherein A-----B is absent.

17. The compounds according to claim 1 selected from the group consisting of: (+)-(3S,4S)-6,6-Dimethyl-(1,1-dimethylheptyl)-1-hydroxy-9-(imidazolomethyl)-6a,7,10,10a- tetrahydro-6H- dibenzo[b,d]pyran; (+)-(3S,4S)-6,6-Dimethyl-(1,1-dimethylheptyl)-1-hydroxy-9-(pyrazolomethyl)-6a,7,10,10a- tetrahydro-6H-dibenzo[b,d]pyran; (+)-(3S,4S)-6,6-Dimethyl-(1,1-dimethylheptyl)-1-hydroxy-9-(1H-imidazol-2-ylsulfanyl methyl)-6a,7,10,10a- tetrahydro-6H- dibenzo[b,d]pyran; (+)-(3S,4S)-6,6-Dimethyl-(1,1-dimethylheptyl)-1-hydroxy-9-(4-piperidinopiperidine methyl) 6a,7,10,10a- tetrahydro-6H- dibenzo[b,d]pyran; (+)-(3S,4S)-6,6-Dimethyl-(1,1-dimethylheptyl)-1-hydroxy-9-(4-methylpiperidine methyl)-6a,7,10,10a- tetrahydro-6H- dibenzo[b,d]pyran.

18. A pharmaceutical composition comprising as an active ingredient is a compound of the general formula (I):



having the (3S,4S) configuration and being essentially free of the (3R,4R) enantiomer, wherein A-----B indicates an optional 1(2) or 6(1) double bond,

$\mathbf{R_1}$ is

A) R3 where R3 is selected from the group consisting of

a) a linear or branched, saturated or unsaturated, carbon side chain comprising 1-8 carbon atoms interrupted by 1-3 heteroatoms; or

b) a saturated or unsaturated cyclic moiety, an aromatic moiety or a heterocyclic moiety; the cyclic moiety having from 5-20 atoms comprising one or two-ringed structures, wherein each ring comprises 3-8 carbons, interrupted by 1-4 heteroatoms, said heteroatoms each independently selected from the group consisting of N, O, and S; wherein each ring optionally is further substituted with one or more groups selected from

i) C_{1-6} alkyl,

ii) C_{1-6} alkoxy,

iii) C_{1-6} alkylthio,

iv) Halo,

v) Carboxyl

vi) $-CO_2-C_{1-4}$ alkyl

vii) keto,

viii) nitro,

ix) a saturated or unsaturated cyclic moiety, or an aromatic or a heterocyclic moiety as defined in b above;

B) an amine or an amide substituted with at least one substituent as defined in R3 above;

C) a thiol, a sulfide, a sulfoxide, a sulfone, a thioester or a thioamide optionally substituted with one substituent as defined in R3 above;

D) an ether –OR3 wherein R3 is as defined above;

G is (a) halogen, (b) C_1-C_5 alkyl, or (c) -OR wherein R is (a') -R", wherein R" is hydrogen or C_1-C_5 alkyl optionally containing a terminal -OR'" or -OC(O)R'" moiety wherein R'" is hydrogen or C_1-C_5 alkyl, or (b') -C(O)R'" wherein R'" is as previously defined,

and $\mathbf{R_2}$ is (a) C_1-C_{12} alkyl, (b) -OR"", in which R"" is a straight chain or branched C_2-C_9 alkyl which may be substituted at the terminal carbon atom by a phenyl group, or (c) $-(CH_2)_nOR'''$ wherein n is an integer of 1 to 7 and R'" is hydrogen or C_1-C_5 alkyl,

with the proviso that $\mathbf{R_1}$ is other than a heterocyclic moiety having a labile hydrogen atom so that said moiety acts as a carboxylic acid analogue.

19. The composition according to claim 18 wherein $\mathbf{R_1}$ is a saturated or unsaturated cyclic moiety, an aromatic moiety or a heterocyclic moiety having from 5-20 atoms comprising one or two-ringed structures, wherein each ring comprises 3-8 carbons interrupted by 0-4 heteroatoms, said heteroatoms in each ring independently selected from the group consisting of N, O, and S; optionally further substituted with at least one substituent selected from the group consisting of lower alkyl, halogen, nitro, cyano, -SR'", -NHR'", $-N(R''')_2$, -OR'", -COR'" , -C(O)OR'" or NH-COR'" moiety wherein R'" is hydrogen or C_1-C_5 alkyl.

20. The composition according to claim 18 wherein $\mathbf{R_1}$ is a heterocyclic moiety selected from the group consisting of an imidazolyl, an imidazolinyl, a morpholino, a piperidyl, a piperazinyl, a pyrazolyl, a pyrrolyl, a pyrrolidinyl, a triazolyl,

and a tetrazolyl, the heterocyclic moiety optionally further substituted wherein the substituent is selected from the group consisting of C_{1-6} alkyl, C_{1-6} alkyloxy, C_{1-6} alkylthio, keto, carboxy , or nitro.

21. The composition according to claim 18 wherein $\mathbf{R_1}$ is imidazolyl.

22. The composition according to claim 18 wherein $\mathbf{R_1}$ is pyrazolyl.

23. The composition of claim 18 where $\mathbf{R_1}$ is 2-methyl thio-2-imidazolinyl.

24. The composition of claim 18 where $\mathbf{R_1}$ is 4-methylpiperidine.

25. The composition according to claim 18 wherein A-----B is a 6(1) double bond, and G is –OH, or lower acyloxy.

26. The composition according to claim 25 wherein $\mathbf{R_2}$ is 1,1-dimethylheptyl or 1,2-dimethylheptyl.

27. The composition of claim 26 wherein $\mathbf{R_1}$ is selected from the group consisting of imidazole, pyrazole, oxazole, isoxazole, tetrahydropyridine, pyrazoline, oxazoline, pyrrolidine, imidazoline, 2-thio-imidazole, 2-methylthio-imidazoline, 4-methyl-2-imidazoline, 4,4-dimethyl-2-imidazoline, methyl sulfide, methylsulfoxide, acetamido, benzamide, cyano, 1,2,4-triazole, 1,3,4-triazole, 1,2,3,4-tetrazole, 1,2,3,5-tetrazole, thiophene, phenyl, morpholine, thiomorpholine, thiazolidine, glycerol, piperazine, piperidine, 4-methylpiperidine and tetrahydropyran, the heterocyclic moiety optionally further substituted wherein the substituent is selected from the group consisting of C_{1-6} alkyl, C_{1-6} alkyloxy, C_{1-6} alkylthio, keto, carboxy , or nitro.

28. The composition of claim 27 wherein $\mathbf{R_1}$ is imidazole.

29. The composition of claim 27 wherein $\mathbf{R_1}$ is pyrazole.

30. The composition of claim 27 wherein R_1 is 2-methyl thio-2-imidazoline.

31. The composition of claim 27 wherein R_1 is 4-methylpiperidine.

32. The composition according to claim 27 wherein G is –OH, or lower acyloxy.

33. The composition according to claim 32 wherein A-----B is absent.

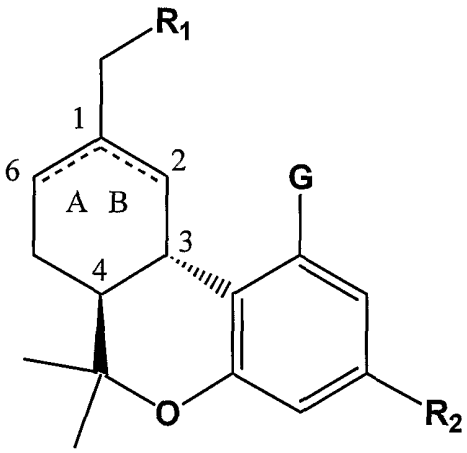
34. The composition according to claim 18 wherein the compound of general formula (I) is selected from group consisting of (+)-(3S,4S)-6,6-Dimethyl-(1,1-dimethylheptyl)-1-hydroxy-9-(imidazolomethyl)-6a,7,10,10a- tetrahydro-6H-dibenzo[b,d]pyran;, (+)-(3S,4S)-6,6-Dimethyl-(1,1-dimethylheptyl)-1-hydroxy-9-(pyrazolomethyl)-6a,7,10,10a- tetrahydro-6H- dibenzo[b,d]pyran;, (+)-(3S,4S)-6,6-Dimethyl-(1,1-dimethylheptyl)-1-hydroxy-9-(1H-imidazol-2-ylsulfanyl methyl)-6a,7,10,10a- tetrahydro-6H- dibenzo[b,d]pyran; (+)-(3S,4S)-6,6-Dimethyl-(1,1-dimethylheptyl)-1-hydroxy-9-(4-piperidinopiperidine methyl) 6a,7,10,10a- tetrahydro-6H- dibenzo[b,d]pyran; (+)-(3S,4S)-6,6-Dimethyl-(1,1-dimethylheptyl)-1-hydroxy-9-(4-methylpiperidine methyl)-6a,7,10,10a- tetrahydro-6H- dibenzo[b,d]pyran.

35. The composition of claim 18 wherein the carrier or diluent is an aqueous cosolvent solution comprising a pharmaceutically acceptable cosolvent, a micellar solution prepared with natural or synthetic ionic or non-ionic surfactants, or a combination of such cosolvent and micellar solutions.

36. The composition of claim 35 wherein the carrier comprises a solution of ethanol, a surfactant, and water.

37. The composition of claim 35 wherein the carrier is an emulsion comprising a triglyceride, lecithin, glycerol, an emulsifier, an antioxidant, and water.

38. A method for treating or preventing inflammatory diseases or disorders, damage resulting from ischemia, injuries to the central nervous system and neurodegenerative disorders, pain, autoimmune diseases by administering to a patient a therapeutically effective amount of a pharmaceutical composition comprising a compound of the formula (I):



having the (3S,4S) configuration and being essentially free of the (3R,4R) enantiomer, wherein A-----B indicates an optional 1(2) or 6(1) double bond,

R_1 is

A) R3 where R3 is selected from the group consisting of

a) a linear or branched, saturated or unsaturated, carbon side chain comprising 1-8 carbon atoms interrupted by 1-3 heteroatoms; or

b) a saturated or unsaturated cyclic moiety, an aromatic moiety or a heterocyclic moiety; the cyclic moiety having from 5-20 atoms comprising one or two-ringed structures, wherein each ring comprises 3-8 carbons, interrupted by 1-4 heteroatoms, said heteroatoms each independently selected from the group consisting of N, O, and S; wherein each ring optionally is further substituted with one or more groups selected from

i) C_{1-6} alkyl,

ii) C_{1-6} alkoxy,

iii) C_{1-6} alkylthio,

iv) Halo,

v) Carboxyl

vi) $-CO_2-C_{1-4}$ alkyl

vii) keto,

viii) nitro,

ix) a saturated or unsaturated cyclic moiety, or an aromatic or a heterocyclic moiety as defined in b above;

B) an amine or an amide substituted with at least one substituent as defined in R3 above;

C) a thiol, a sulfide, a sulfoxide, a sulfone, a thioester or a thioamide optionally substituted with one substituent as defined in R3 above;

D) an ether –OR3 wherein R3 is as defined above;

G is (a) halogen, (b) C_1-C_5 alkyl, or (c) -OR wherein R is (a') -R", wherein R" is hydrogen or C_1-C_5 alkyl optionally containing a terminal -OR'" or -OC(O)R'" moiety wherein R'" is hydrogen or C_1-C_5 alkyl, or (b') -C(O)R'" wherein R'" is as previously defined,

and $\mathbf{R_2}$ is (a) C_1-C_{12} alkyl, (b) -OR"", in which R"" is a straight chain or branched C_2-C_9 alkyl which may be substituted at the terminal carbon atom by a phenyl group, or (c) $-(CH_2)_nOR'''$ wherein n is an integer of 1 to 7 and R'" is hydrogen or C_1-C_5 alkyl;

with the proviso that $\mathbf{R_1}$ is other than a heterocyclic moiety having a labile hydrogen atom so that said moiety acts as a carboxylic acid analogue.

39. The method according to claim 38 wherein $\mathbf{R_1}$ is a saturated or unsaturated cyclic moiety, an aromatic moiety or a heterocyclic moiety having from 5-20 atoms comprising one or two-ringed structures, wherein each ring comprises 3-8 carbons interrupted by 0-4 heteroatoms, said heteroatoms in each ring independently selected from the group consisting of N, O, and S; optionally further substituted with at least one substituent selected from the group consisting of lower alkyl, halogen, nitro, cyano, -

SR'", -NHR'", -N(R'")$_2$, -OR'", -COR'" , -C(O)OR'" or NH-COR'" moiety wherein R'" is hydrogen or C_1-C_5 alkyl.

40. The method according to claim 38 wherein $\mathbf{R_1}$ is a heterocyclic moiety selected from the group consisting of an imidazolyl, an imidazolinyl, a morpholino, a piperidyl, a piperazinyl, a pyrazolyl, a pyrrolyl, a pyrrolidinyl, a triazolyl, and a tetrazolyl, the heterocyclic moiety optionally further substituted wherein the substituent is selected from the group consisting of C_{1-6} alkyl, C_{1-6} alkyloxy, C_{1-6} alkylthio, keto, carboxy , or nitro.

41. The method according to claim 38 wherein $\mathbf{R_1}$ is imidazolyl.

42. The method according to claim 38 wherein $\mathbf{R_1}$ is pyrazolyl.

43. The method of claim 38 where $\mathbf{R_1}$ is 2-methyl thio-2-imidazolinyl.

44. The method of claim 38 where $\mathbf{R_1}$ is 4-methylpiperidine.

45. The method according to claim 38 wherein A-----B is a 6(1) double bond, and G is –OH, or lower acyloxy.

46. The method according to claim 45 in which and $\mathbf{R_2}$ is 1,1-dimethylheptyl or 1,2-dimethylheptyl.

47. The method of claim 46 wherein $\mathbf{R_1}$ is selected from the group consisting of imidazole, pyrazole, oxazole, isoxazole, tetrahydropyridine, pyrazoline, oxazoline, pyrrolidine, imidazoline, 2-thio-imidazole, 2-methylthio-imidazoline, 4-methyl-2-imidazoline, 4,4-dimethyl-2-imidazoline, methyl sulfide, methylsulfoxide, acetamido, benzamide, cyano, 1,2,4-triazole, 1,3,4-triazole, 1,2,3,4-tetrazole, 1,2,3,5-tetrazole, thiophene, phenyl, morpholine, thiomorpholine, thiazolidine, glycerol, piperazine, piperidine, 4-methylpiperidine and tetrahydropyran, the heterocyclic moiety optionally

further substituted wherein the substituent is selected from the group consisting of C_{1-6} alkyl, C_{1-6} alkyloxy, C_{1-6} alkylthio, keto, carboxy , or nitro.

48. The method of claim 47 wherein $\mathbf{R_1}$ is imidazole.

49. The method of claim 47 wherein $\mathbf{R_1}$ is pyrazole.

50. The method of claim 47 wherein $\mathbf{R_1}$ is 2-methyl thio-2-imidazoline.

51. The method of claim 47 wherein $\mathbf{R_1}$ is 4-methylpiperidine

52. The method according to claim 47 wherein G is –OH, or lower acyloxy.

53. The method according to claim 52 wherein A-----B is absent.

54. The method s according to claim 38 selected from the list which may be referred to as (+)-(3S,4S)-6,6-Dimethyl-(1,1-dimethylheptyl)-1-hydroxy-9-(imidazolomethyl)-6a,7,10,10a- tetrahydro-6H- dibenzo[b,d]pyran, (+)-(3S,4S)-6,6-Dimethyl-(1,1-dimethylheptyl)-1-hydroxy-9-(pyrazolomethyl)-6a,7,10,10a- tetrahydro-6H- dibenzo[b,d]pyran, (+)-(3S,4S)-6,6-Dimethyl-(1,1-dimethylheptyl)-1-hydroxy-9-(1H-imidazol-2-ylsulfanyl methyl)-6a,7,10,10a- tetrahydro-6H- dibenzo[b,d]pyran, (+)-(3S,4S)-6,6-Dimethyl-(1,1-dimethylheptyl)-1-hydroxy-9-(4-piperidinopiperidine methyl) 6a,7,10,10a- tetrahydro-6H- dibenzo[b,d]pyran, (+)-(3S,4S)-6,6-Dimethyl-(1,1-dimethylheptyl)-1-hydroxy-9-(4-methylpiperidine methyl)-6a,7,10,10a- tetrahydro-6H- dibenzo[b,d]pyran.

55. The method of claim 38 which comprises administering said composition to a patient in order to protect against excitatory amino acid-mediated neurotoxicity.

56. The method of claim 38 which comprises administering said composition to a patient who exhibits the symptoms associated with neural injury due to edema,

neural injury due to cerebral ischemia, neural injury due to head trauma, poisoning of the central nervous system, cardiac arrest, stroke, optic neuropathy, or spinal cord injury.

57. The method of claim 38 which comprises administering said composition to a patient who exhibits the symptoms associated with epilepsy, Huntington's disease, Parkinson's disease, amyotrophic lateral sclerosis or Alzheimer's disease.

58. A method for blocking or preventing pain, including chronic and neuropathic pain, or inflammation in a patient which comprises administering to said patient, a therapeutically effective amount of a pharmaceutical composition according to claim 18.

59. A method according to claim 38 or claim 58 wherein the daily dosage of said compound is between 0.01 and 25 mg/kg.

60. Use for the preparation of a neuroprotective medicament for treating neurological diseases, traumatic injury to central nervous system, ischemic injury to the nervous system, degenerative diseases of the nervous system, inflammatory disease of the central nervous system, of a compound according to any one of claims 1-17.

61. Use for the preparation of an anti-inflammatory or analgesic medicament for treating or preventing inflammatory diseases and/or autoimmune diseases including multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosis, Graves disease, Hashimoto's thyroiditis, inflammatory bowel disease of a compound according to any one of claims 1-17.

62. Use for the preparation of a medicament for treating, alleviating or preventing myocardial infarction, atheroma, unstable angina, restenosis, or ischemic damage to the cardiovascular system of a compound according to any one of claims 1-17.

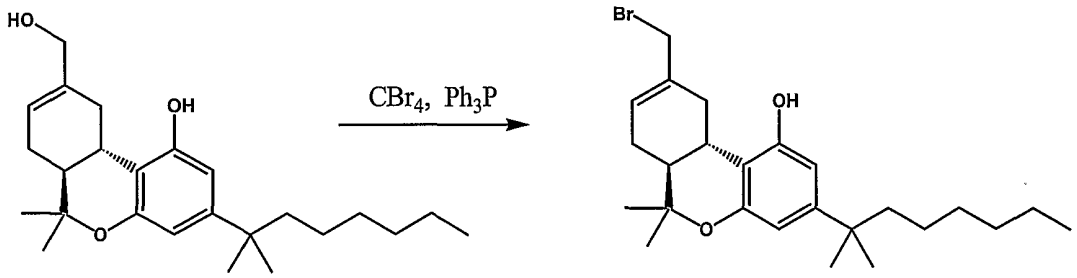
63. Use for the preparation of a medicament for treating or alleviating ischemic and/or inflammatory damage to body organs including the lungs, liver, kidney or joints related to diseases such as pulmonary, hepatic or renal ischemias, or rheumatoid arthritis or septic shock of a compound according to any one of claims 1-17.

5

64. Use for the preparation of a medicament for treating or preventing glaucoma, retinal degeneration or emesis of a compound according to any one of claims 1-17.

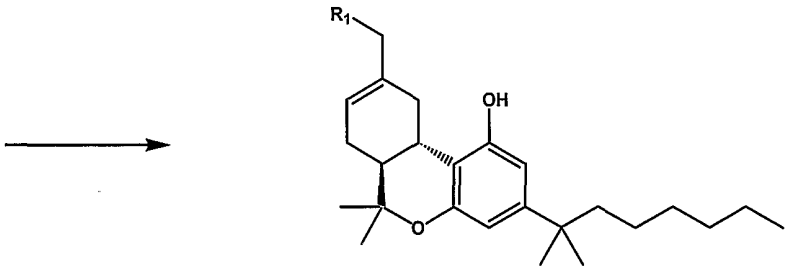
10

Figure 1



Dexanabinol

Compound I



Example of new analogs

Figure 2

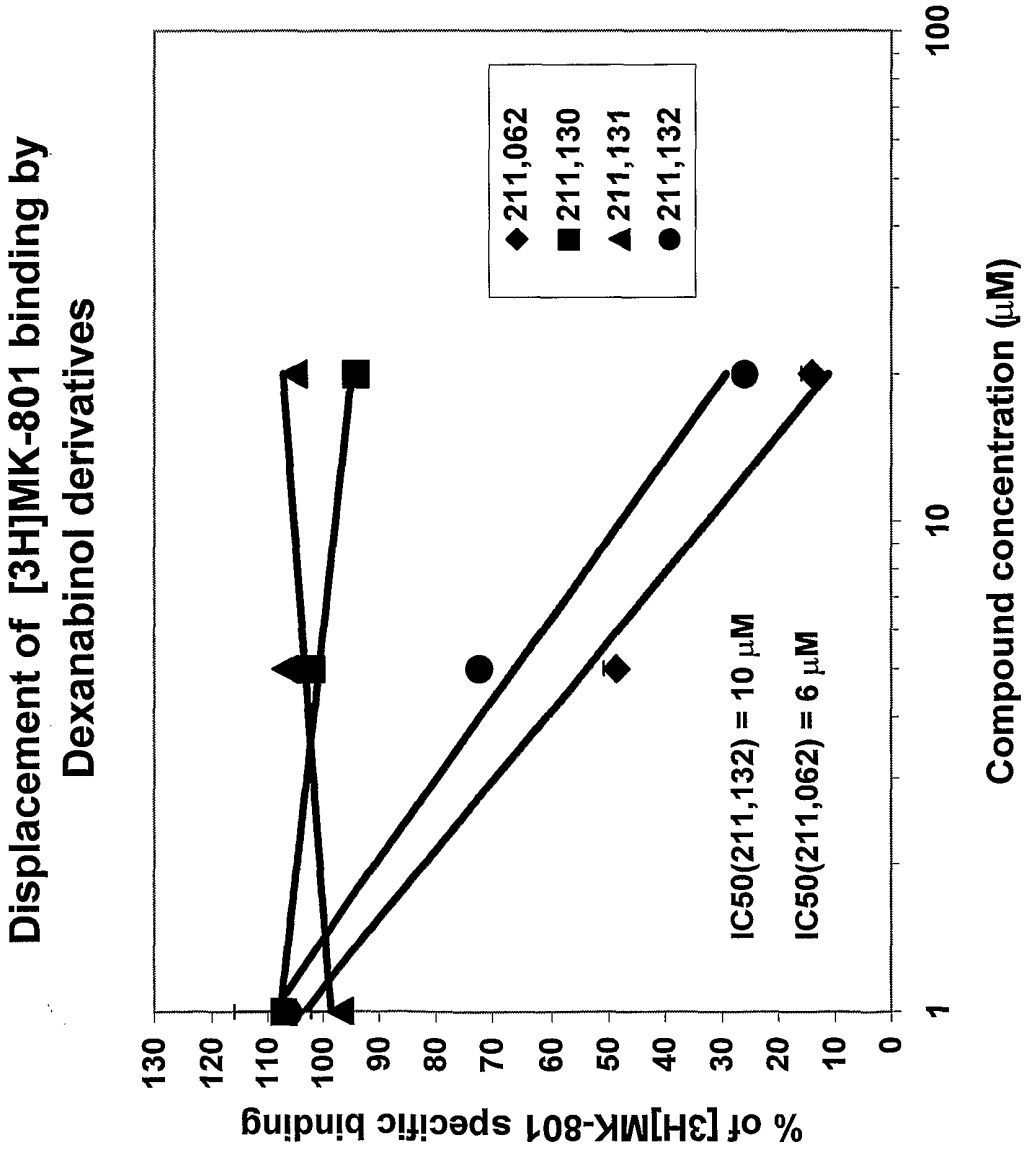

Displacement of [3H]MK-801 binding by Dexanabinol derivatives
% of [3H]MK-801 specific binding
Compound concentration (μM)
211,062
211,130
211,131
211,132
IC50(211,132) = 10 μM
IC50(211,062) = 6 μM
130
120
110
100
90
80
70
60
50
40
30
20
10
0
1
10
100

Figure 3

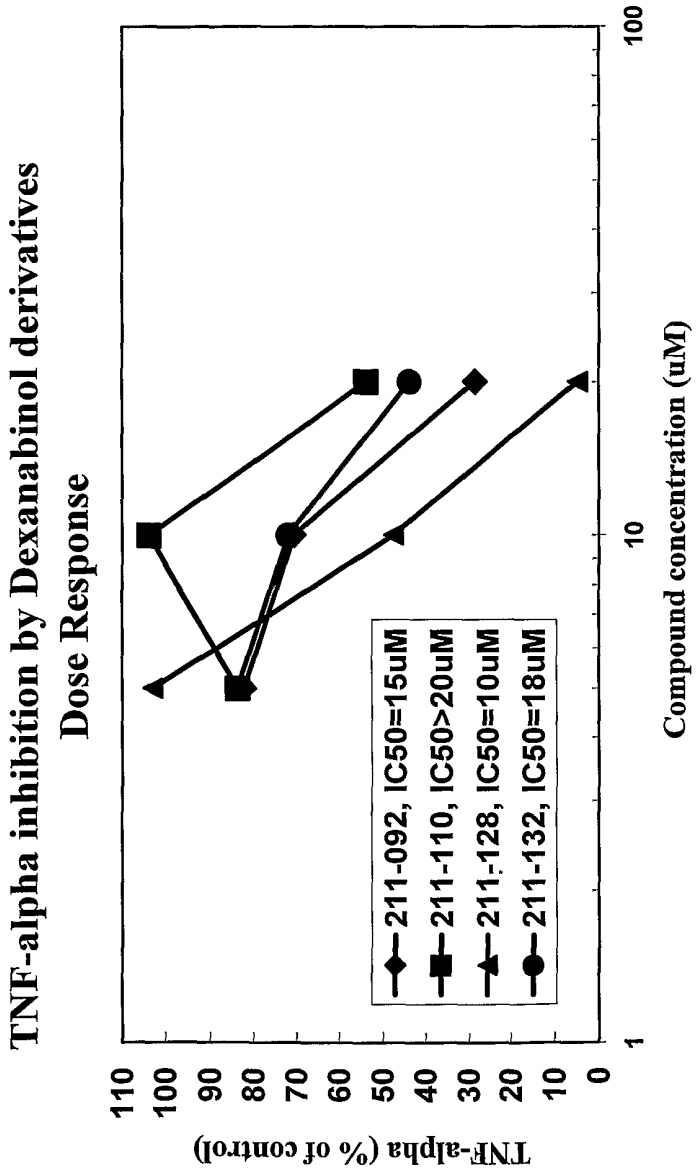

TNF-alpha inhibition by Dexanabinol derivatives
Dose Response
TNF-alpha (% of control)
110
100
90
80
70
60
50
40
30
20
10
0
1
10
100
Compound concentration (uM)
211-092, IC50=15uM
211-110, IC50>20uM
211-128, IC50=10uM
211-132, IC50=18uM

Figure 4

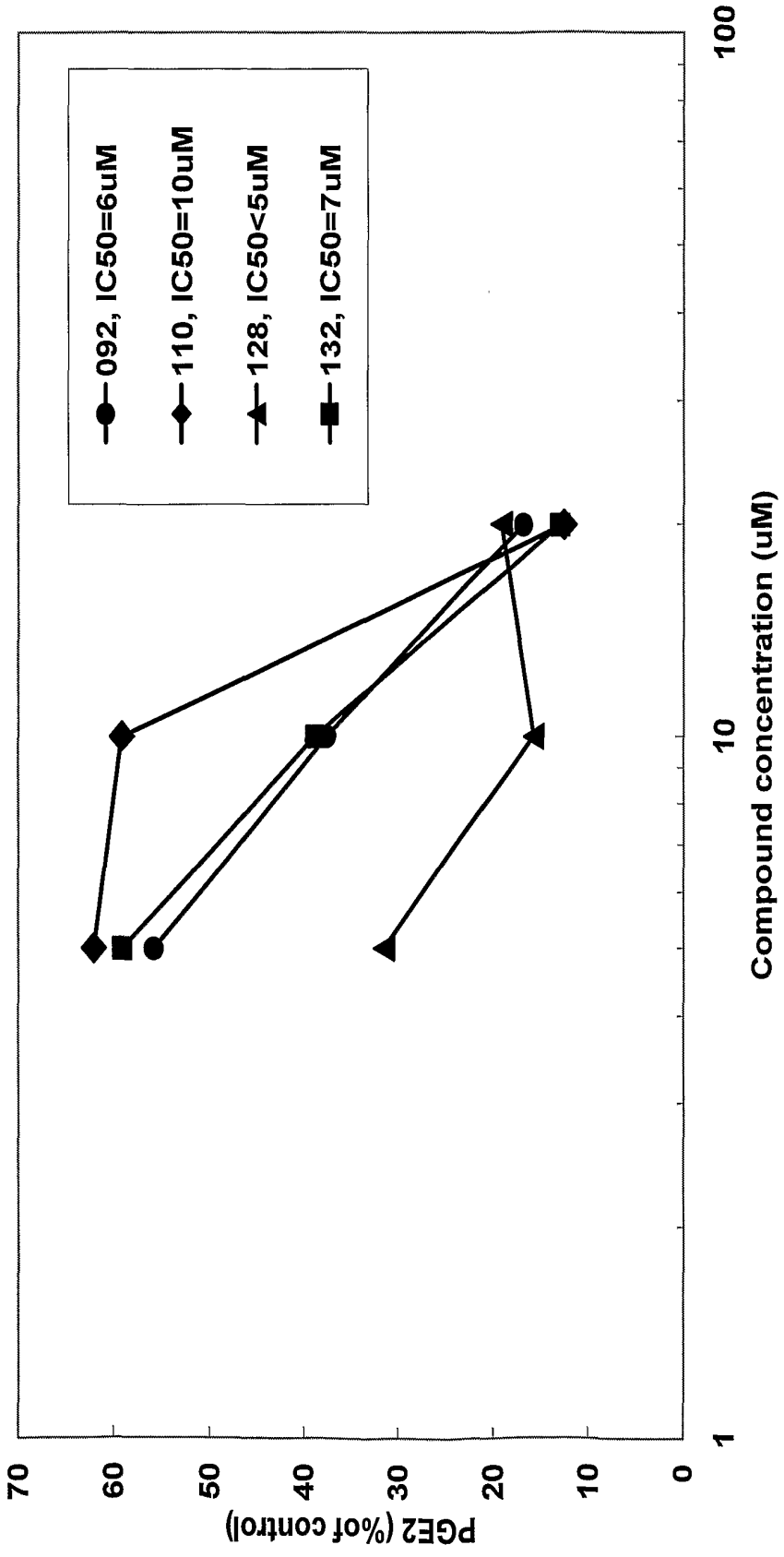

PGE2 Inhibition by Dexanabinol Analogs
Dose Response
092, IC50=6uM
110, IC50=10uM
128, IC50<5uM
132, IC50=7uM
PGE2 (%of control)
0
10
20
30
40
50
60
70
1
10
100
Compound concentration (uM)

Figure 5

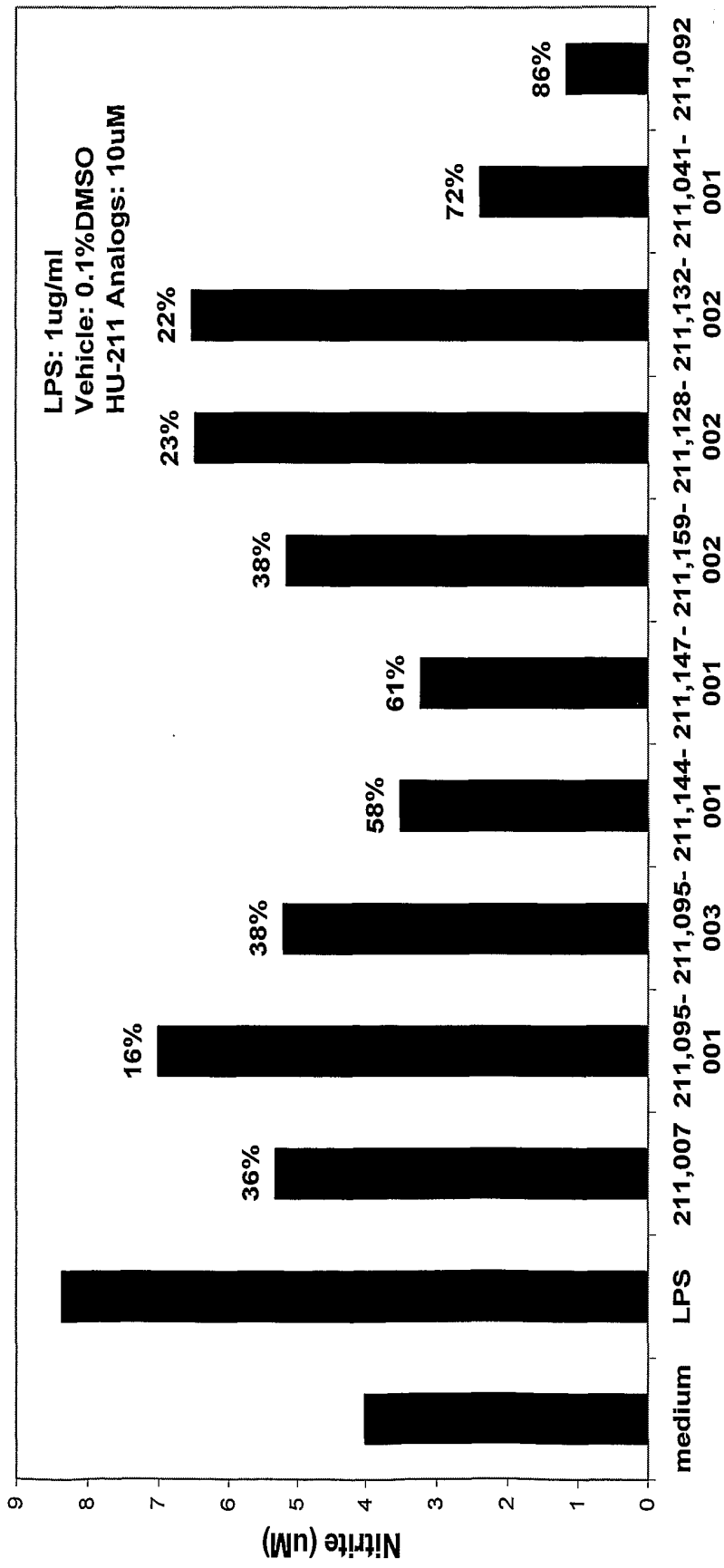

Nitric Oxide Synthase Inhibition by Dexanabinol Analogs
In Supernatants of LPS-Stimulated Macrophages
LPS: 1ug/ml
Vehicle: 0.1%DMSO
HU-211 Analogs: 10uM
Nitrite (uM)
0
1
2
3
4
5
6
7
8
9
16%
36%
38%
58%
61%
38%
23%
22%
72%
86%
medium
LPS
211,007
211,095-001
211,095-003
211,144-001
211,147-001
211,159-002
211,128-002
211,132-002
211,041-001
211,092

Figure 6

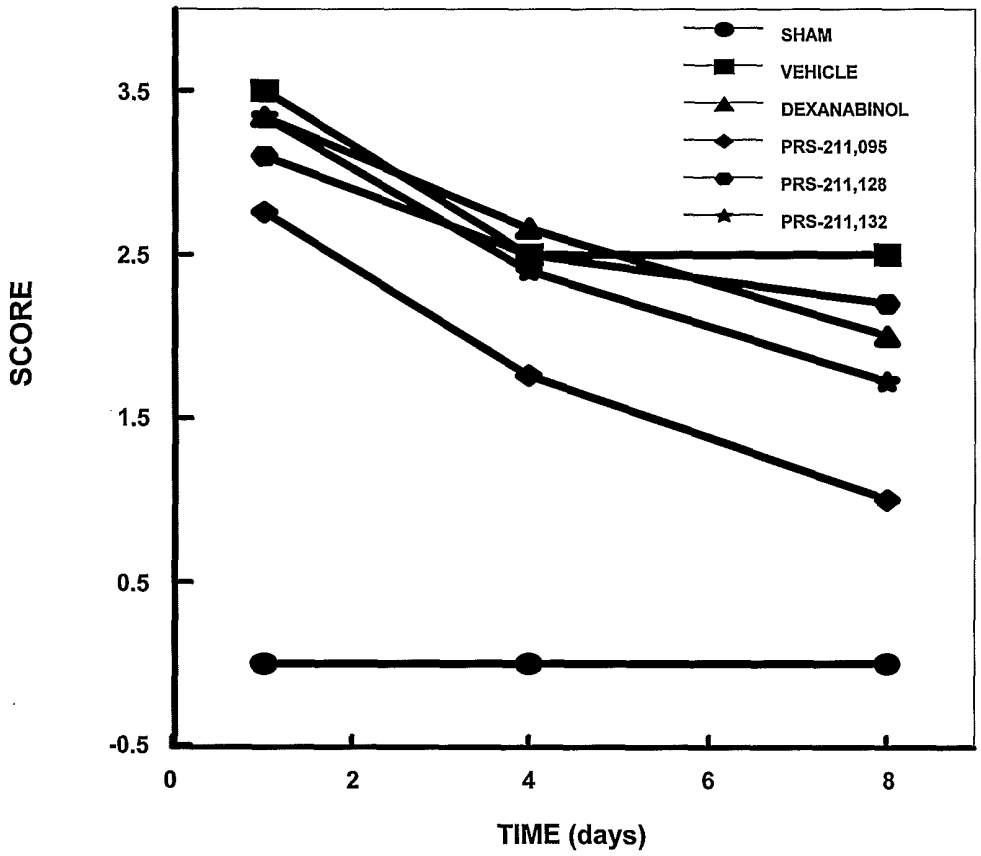

SHAM
VEHICLE
DEXANABINOL
PRS-211,095
PRS-211,128
PRS-211,132
SCORE
3.5
2.5
1.5
0.5
-0.5
0
2
4
6
8
TIME (days)

Figure 7

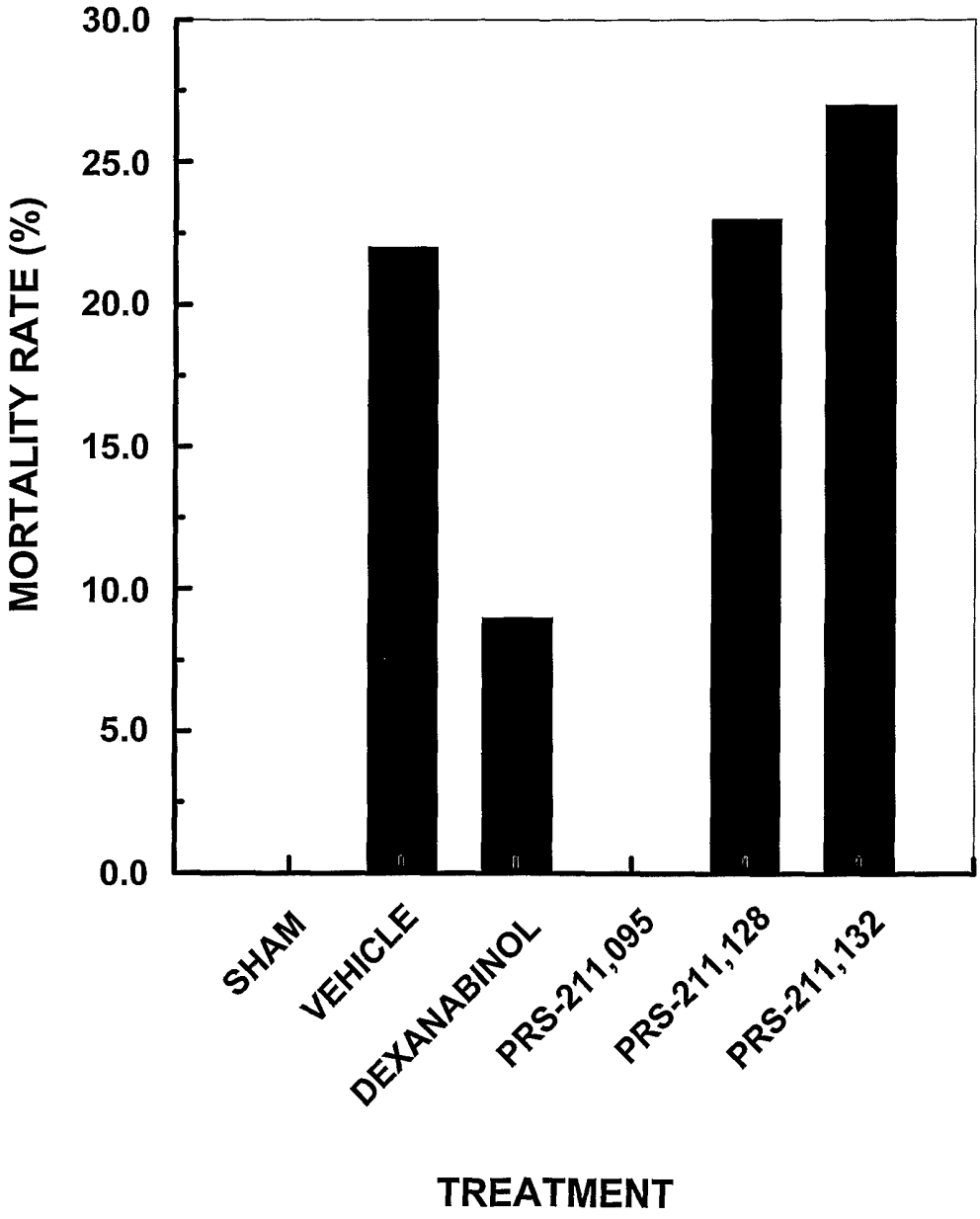

30.0
25.0
20.0
15.0
10.0
5.0
0.0
MORTALITY RATE (%)
SHAM
VEHICLE
DEXANABINOL
PRS-211,095
PRS-211,128
PRS-211,132
TREATMENT

Figure 8

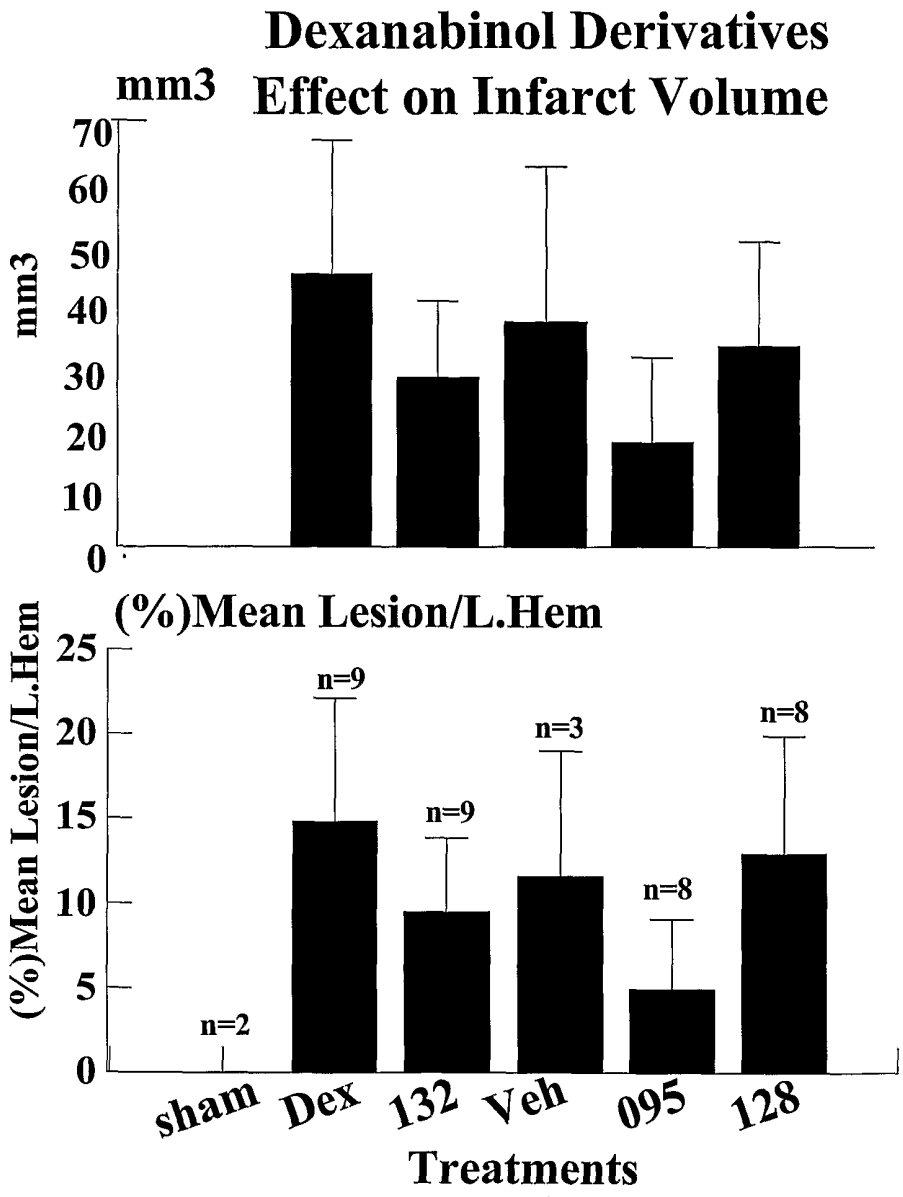

Dexanabinol Derivatives
Effect on Infarct Volume
mm3
70
60
50
40
30
20
10
0
(%)Mean Lesion/L.Hem
25
20
15
10
5
0
n=2
n=9
n=9
n=3
n=8
n=8
sham
Dex
132
Veh
095
128
Treatments

Figure 9

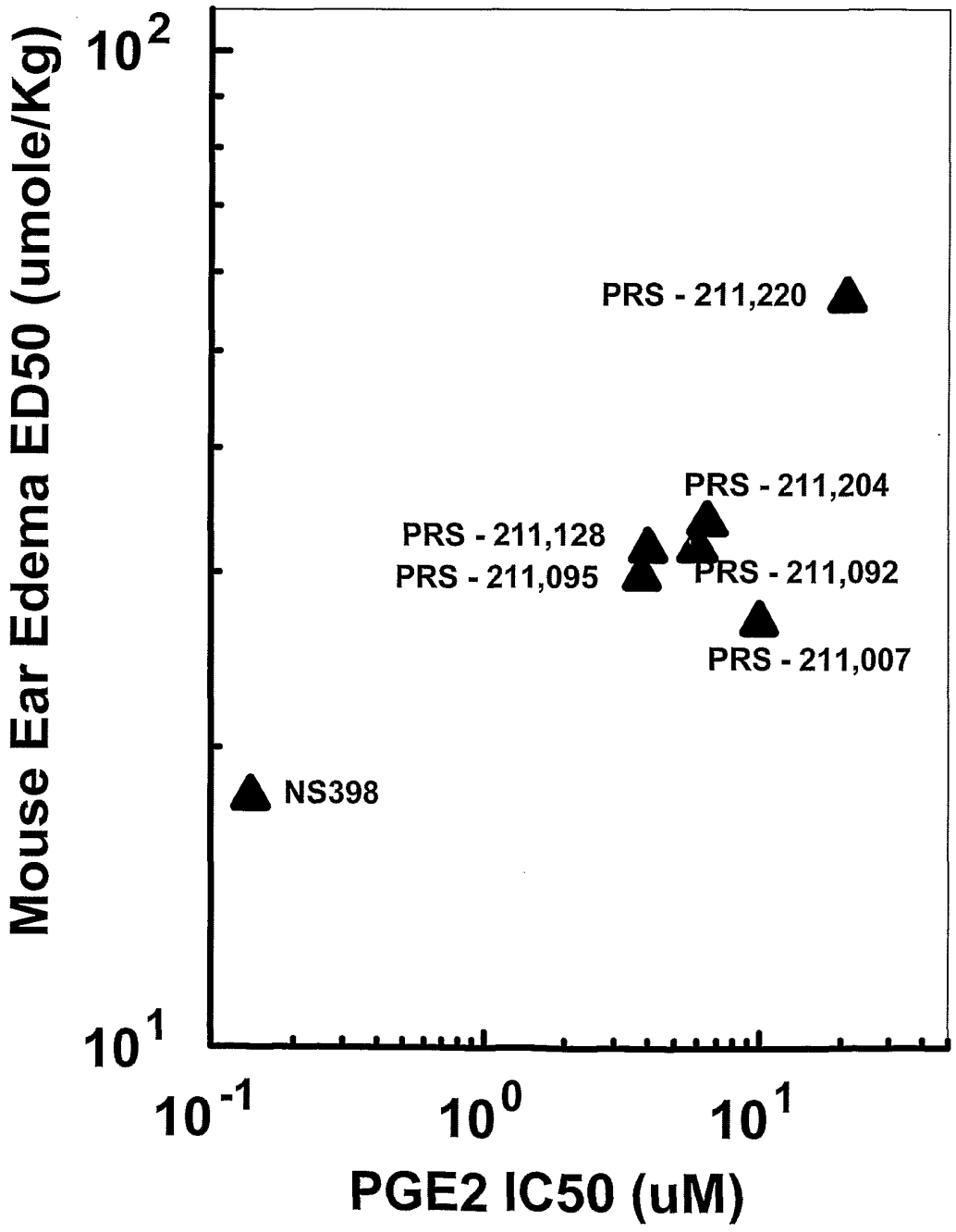

Mouse Ear Edema ED50 (umole/Kg)
10^2
10^1
10^-1
10^0
10^1
PGE2 IC50 (uM)
PRS - 211,220
PRS - 211,204
PRS - 211,128
PRS - 211,095
PRS - 211,092
PRS - 211,007
NS398

Figure 10

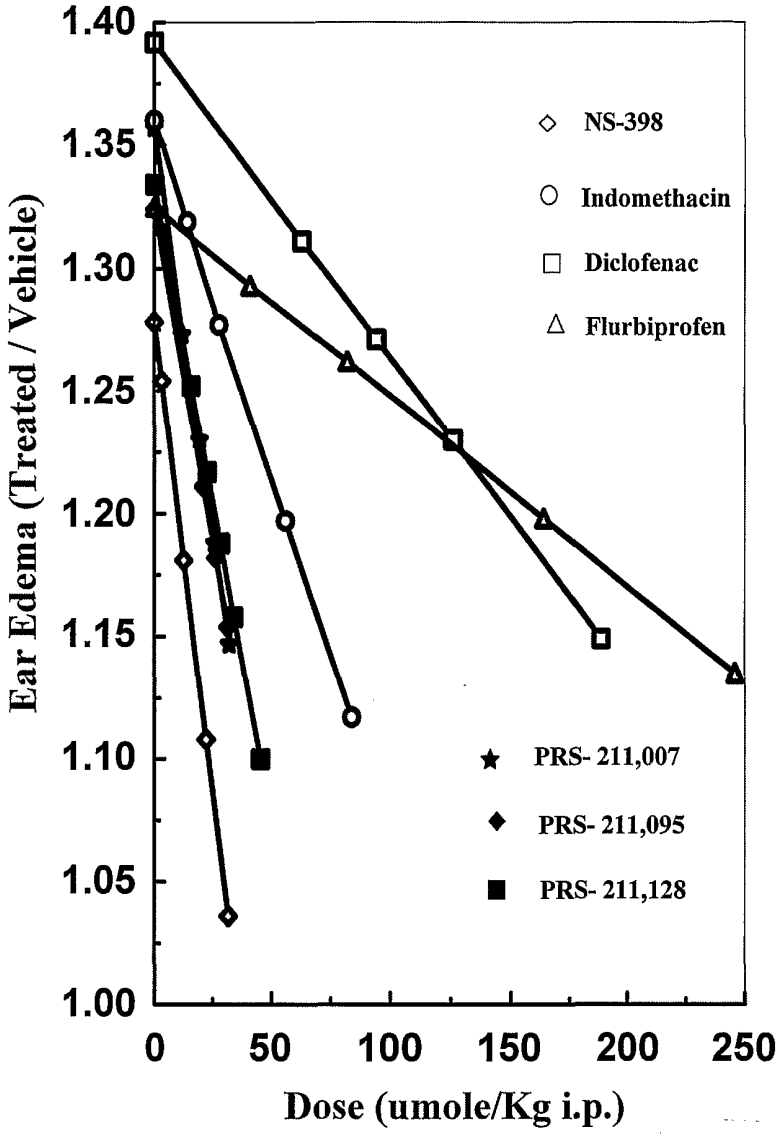

1.40
1.35
1.30
1.25
1.20
1.15
1.10
1.05
1.00
Ear Edema (Treated / Vehicle)
0
50
100
150
200
250
Dose (umole/Kg i.p.)
NS-398
Indomethacin
Diclofenac
Flurbiprofen
PRS- 211,007
PRS- 211,095
PRS- 211,128

Figure 11
CONTRALATERAL PERFORMANCE IN THE STAIRCASE TEST

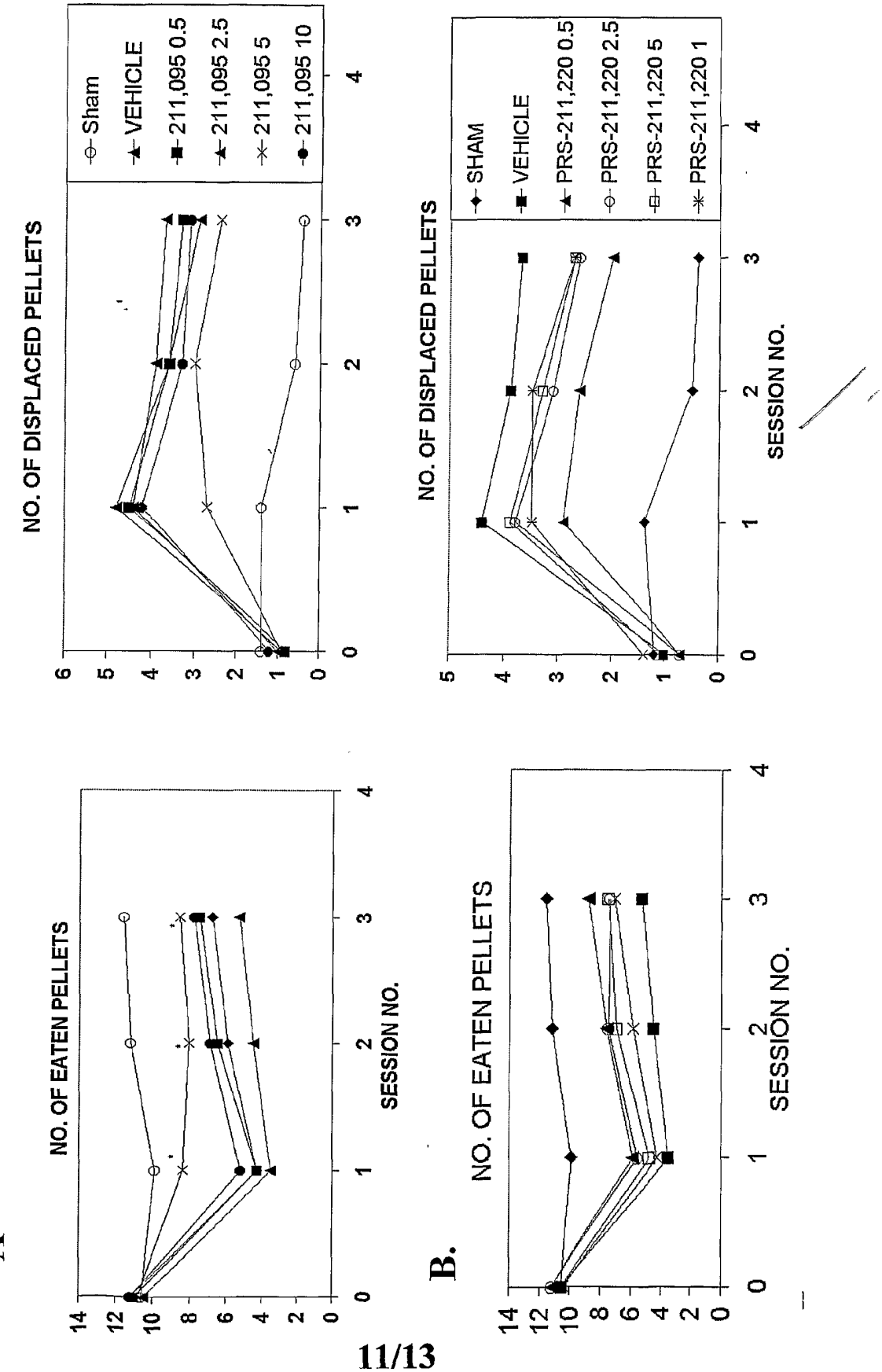


Figure 12
Effect of Dexanabinol Analogs on Infarct size in the Transient Middle Cerebral Artery Occlusion (MCAo) Assay

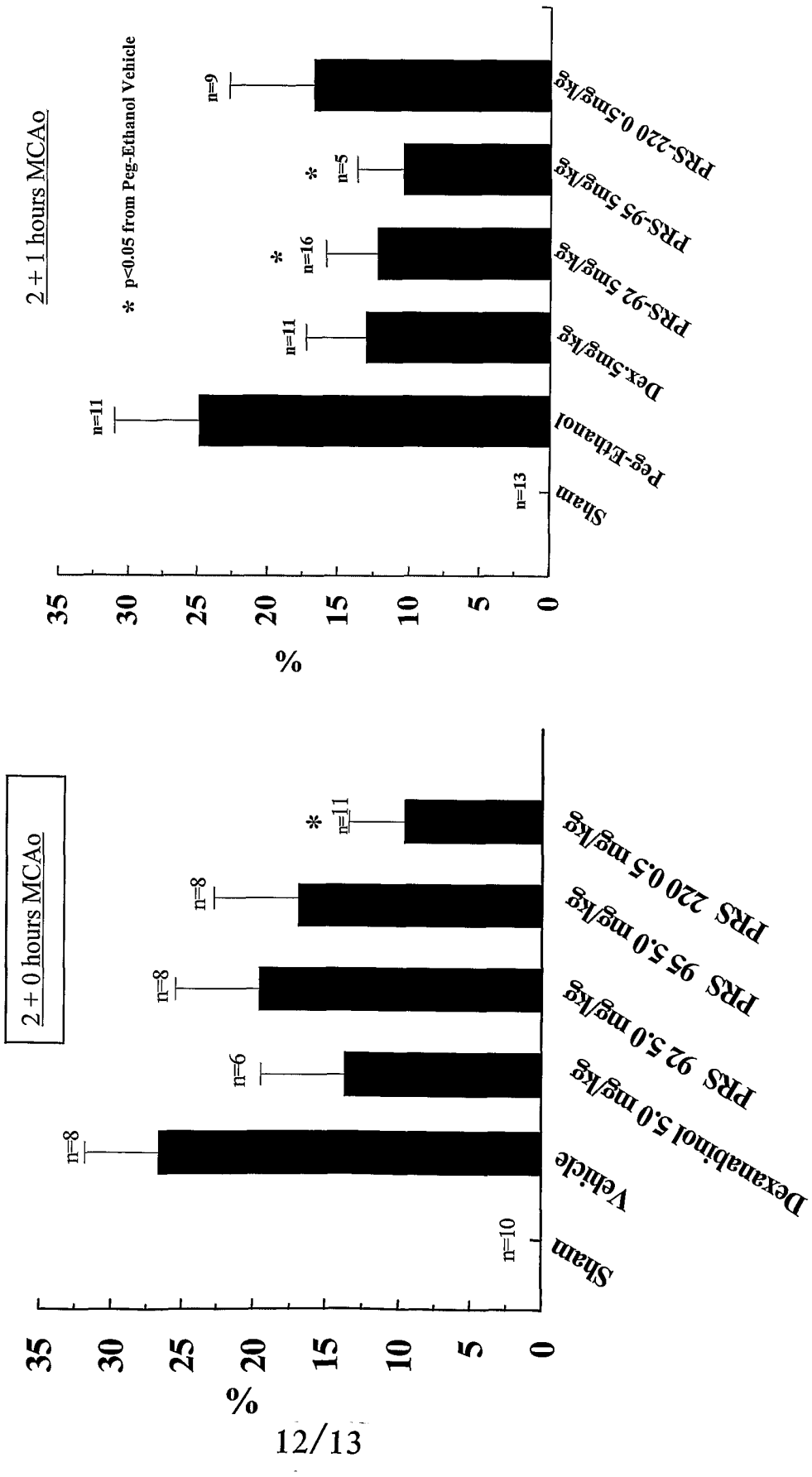
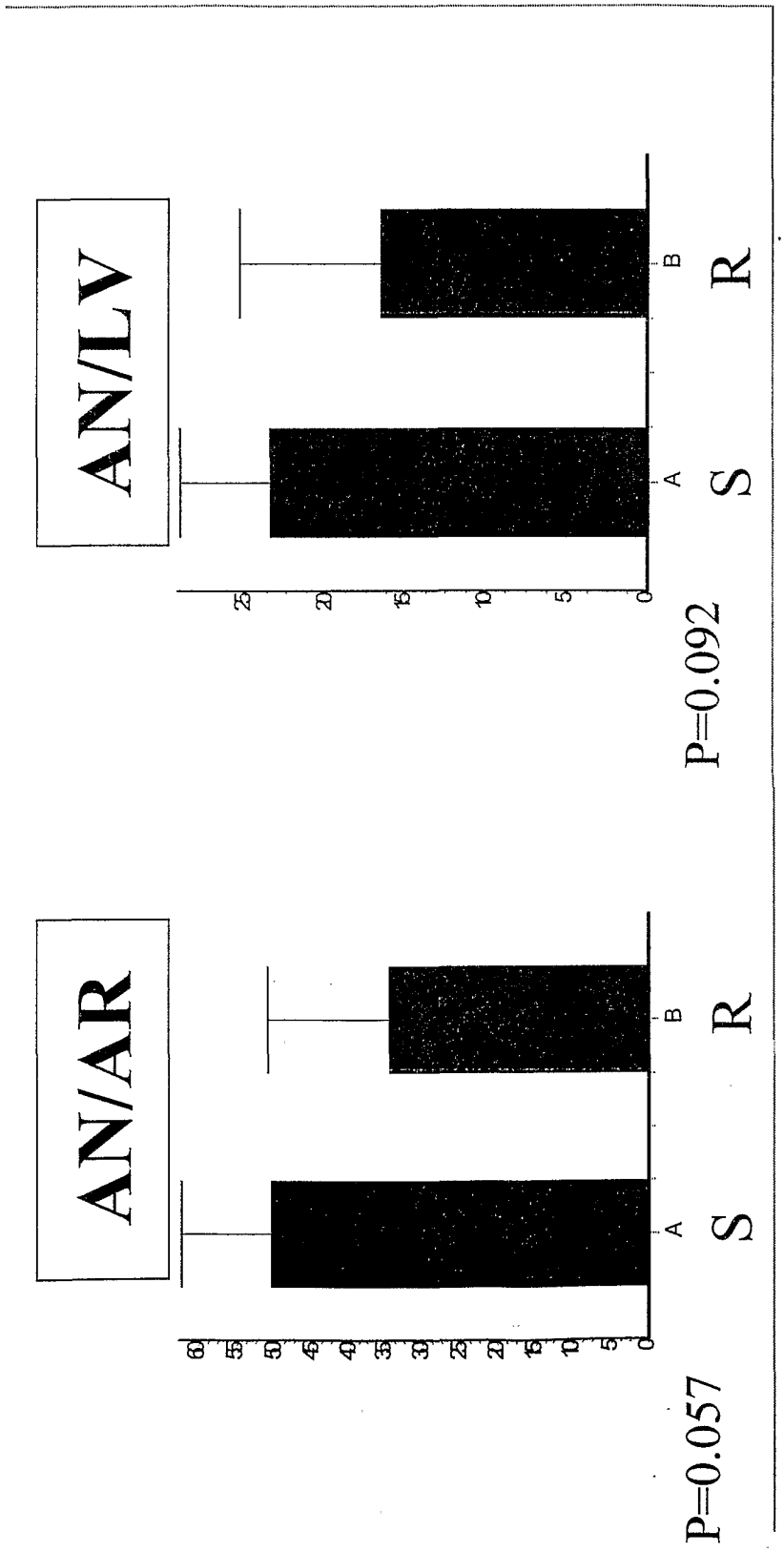


Figure 13
Reduction of Necrotic Area in Dexanabinol Derivative-treated Rats Prior to Myocardial Insult



INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL01/00571

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :C07D 311/80, 233/02; A61K 31/35, 31/415
US CL :549/390; 548/525, 364.4; 514/454, 422, 406
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 549/390; 548/525, 364.4; 514/454, 422, 406

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,872,148 A (MAKRIYANNIS ET AL) 16 FEBRUARY 1999, see abstract.	1-64
A,P	US 6,162,829 A (BURSTEIN) 19 December 2000, cols. 3-4.	1-64
A,P	US 6,166,066 A (MAKRIYANNIS et al) 26 December 2000, cols. 5-6.	1-64
A	US 6,284,788 B1 (MITTENDORF et al) 04 September 2001, cols. 9-10.	1-64

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
28 OCTOBER 2001	19 NOV 2001
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer DEBORAH LAMBKIN Telephone No. (703) 308-1235

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